State of Hawaii
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Forestry and Wildlife
Honolulu, Hawaii 96813

January 13, 2006

Chairperson and Members
Board of Land and Natural Resources
State of Hawaii
Honolulu, Hawaii

Land Board Members:

SUBJECT: REQUEST FOR APPROVAL TO HOLD PUBLIC HEARING ON PROPOSED AMENDMENTS TO HAWAII ADMINISTRATIVE RULES CHAPTER 13-209 (RULES REGULATING ACTIVITIES WITHIN NATURAL AREA RESERVES)

This submittal requests Board approval to hold public hearings on proposed amendments to Hawaii Administrative Rules (HAR) Chapter 13-209 (Rules Regulating Activities within Natural Area Reserves) and to authorize the Chairperson to appoint a hearing master and designate dates and times for the public hearing(s).

BACKGROUND:
HAR Chapter 13-209 were adopted in 1981 pursuant to HRS § 195-5, which authorized the department, with the approval of the Natural Area Reserves Commission, to make rules governing the use, control and protection of areas including within the natural area reserves system. Since that time, the rules were amended only once, as part of a department-wide initiative to specifically prohibit commercial activity without a permit.

Over the years, there have been occasions where shortcomings in the existing administrative rules have resulted in a failure to efficiently or effectively respond to threats to the natural areas reserves. One recent example is the difficulty in preventing poaching in Ahihi-Kinau Natural Area Reserve. While the NARS rules prohibit the removal of animal life from a reserve, the rules do not prohibit possession of fishing equipment within a reserve. As a result, the department cannot prevent harm, but must wait until someone has already caught fish before citing the illegal activity. Thus, one of the proposed amendments is to change 13-209 to add a provision prohibiting fishing equipment, similar to those already adopted for marine life conservation districts.

The need to comprehensively review Chapter 13-209 and propose amendments to strengthen the department's ability to protect the unique natural and cultural features of the NARS system, has been recommended for some time by both staff and NARS Commissioners. During a planning workshop in late September 2005, staff and Commissioners identified priority rule changes necessary to ensure preservation of the unique natural resources. Based on those discussions and further input from NARS staff and from the Rules and Policy Subcommittee of the NARS Commission, the proposed draft amendments (Attachment 1) were developed.

The proposed amendments were presented to the full NARS Commission for review at their December 12, 2005 meeting, where the Commission unanimously voted to forward the proposed amendments (with minor changes that have been incorporated) to the Board for further action.

RULE AMENDMENTS:
The full text of the proposed amendments to HAR Chapter 13-209 is included as Attachment 1. A summary of the proposed changes, including a brief justification for the proposed change, follows:

- Amendment to 13-209-3 (Permitted Activities):
  The proposed changes would remove bedroll camping as a permitted activity and limit hiking and nature study as permitted activities for groups of ten or less. In addition, the proposed change would specify that permitted activities may be limited in closed reserves (or in closed portions of reserves) or in reserves where visiting hours have been established.

These changes are proposed to remove the distinction between bedroll and other camping, a distinction which is not found in other rules of the Division of Forestry and Wildlife. By having the effect of requiring a permit for hiking with groups of more than ten persons in size, the proposed changes would allow better monitoring of large groups and their impact on an individual reserve. Finally, the proposed changes would specify that otherwise permitted activities can be restricted during certain hours or during approved closures.

- Amendment to 13-209-4 (Prohibited Activities):
  The proposed changes would:
- allow service animals accompanying their masters within the NAR;
- specifically prohibit the establishment of residences in the NAR;
- prohibit the possession of tools, equipment, or implements used to take, injure, or kill marine life, plant life, or wildlife;
- prohibit activities in the NAR by groups larger than ten in number;
- prohibit presence in a closed NAR or portion of a NAR or after visiting hours;
- specifically prohibit anchoring in marine waters within a NAR;
- prohibit entry into any cave in a NAR; and
- prohibit actions in a NAR inconsistent with the purpose and intent of the NARS system.

The changes are primarily proposed to enhance enforcement of existing rules and increase the ability of the department to protect the NARS from degradation or damage. For example, preventing the possession of tools used to take marine life would allow DOCARE officers to cite suspected poachers before they violate existing rules against removing marine life. Likewise, preventing anchoring in a NAR would allow DOCARE or resource managers to prevent potential damage to underwater coral, without having to wait for damage to actually occur to the natural resources. The proposed changes to specifically prohibit the establishment of residences in a NAR and to prohibit entry into caves would address shortcomings in the existing rules that were identified during a recent incident of someone attempting to reside within Ahihi-Kinau NAR. In addition, as discussed briefly above, a prohibition on groups larger than ten would allow better monitoring of large groups and their impact on an individual reserve. It should be pointed out that the changes would not institute a blanket prohibition on these additional activities, but that pursuant to the existing rules (HAR § 13-209-5), the board or its authorized representative may issue permits to conduct activities otherwise prohibited. Finally, adding an exception in the existing rules to permit the presence of service animals would remove a barrier that prevents those using service animals from fully experiencing a NARS.

- Addition of 13-209-4.5 (Closing of Areas):
  The proposed changes would specifically authorize the Board or its authorized representative to close or restrict public use of all or any portion of a NAR for up to two years, when deemed necessary by the NARS Commission for the protection of the natural, geological, or cultural resources of a reserve or the safety and welfare of persons or property.

The changes are proposed to give the department another management tool to protect and prevent degradation to the unique natural resources within the NARS and to implement the BLNR policy to protect resources first, to accommodate public use second, and finally where appropriate to allow commercial use. Limiting closure to a period of 2 years ensures regular review of the condition and status of the natural resources and assessment whether continued closure is necessary, so that the public is not unnecessarily excluded from public lands.

- Addition of 13-209-4.6 (Visiting hours):
  The proposed changes would authorize the Board or its authorized representative, with the approval of the Commission, to establish a schedule of visiting hours for individual reserves.

The change is proposed to give the department another management tool to address inappropriate night-time activity within a NAR. Instead of setting visiting hours for the NARS system, the proposed change allows the Board or its authorized representative, with the approval of the Commission, to set a schedule on a reserve by reserve basis, recognizing that a time restriction for one NAR may be inappropriate or unnecessary for another.

- Amendment of 13-209-5 (Special-use permits):
  The proposed changes specify that special use permits are valid for no more than 1 year, outline standard conditions for special-use permits, authorize the addition of special

conditions, require permittees to carry permits with them, state that permits are not transferable, and outline situations where the Board or its authorized representative may revoke or cancel a permit.

The changes were recommended by the Attorney General's office to formalize existing policies into rule, providing permit applicants advance notice of conditions and restrictions on special-use permits.

- Addition of 13-209-5.5 (Applications for Special-use permits)
  The proposed changes outline requirements for special-use permit applications, identify the criteria to be used by the Board or its authorized representative in evaluating special-use permit applications, specify that the burden is on the applicant of demonstrating consistency with the criteria, provide when a public hearing may be held and detail the notice requirements, and set forth a maximum time period for the Board or its authorized representative to render a decision on an application.

The changes were recommended by the Attorney General's office to formalize the permit application process. These changes would provide permit applicants with more complete advance notice of what information is needed by the department to evaluate applications, the criteria used to evaluate applications, and how long the process should take. In addition, these proposed changes would reduce the ability of an unsuccessful applicant to challenge the denial as arbitrary and capricious.

RECOMMENDATION:

That the Board: (1) give approval to proceed with amending HAR Chapter 13-209 as detailed above, including permission to hold public hearings; (2) authorize the Chairperson to appoint hearing master(s) for the respective public hearings; and (3) authorize the Chairperson to set the dates and times for the respective public hearings.

Respectfully submitted,

PAUL J. CONRY, Administrator
Division of Forestry and Wildlife

Attachment 1

APPROVED FOR SUBMITTAL:

PETER T. YOUNG, Chairperson
Board of Land and Natural Resources

AMENDMENTS TO CHAPTER 13-209, HAWAII ADMINISTRATIVE RULES

1. Section 13-209-3, Hawaii Administrative Rules, is amended to read as follows:

"**§ 13-209-3. Permitted activities.**

Hiking[,] and nature study[, and bedroll camping without a tent or other temporary structure] of group size of ten or less are permitted except where restricted pursuant to sections 13-209-4.5 and 13-209-4.6. Hunting is a permitted activity pursuant to hunting rules of the department."
[Effective June 29, 1981; amended ___, 2005]
(Authority: HRS Sec. 195-5) (Implementation: HRS Sec. 195-5)]

2. Section 13-209-4, Hawaii Administrative Rules, is amended to read as follows:

"**§ 13-209-4. Prohibited Activities.**

The following activities are prohibited within a natural area reserve:

(1) To remove, injure, or kill any form of plant or animal life, except game mammals and birds hunted according to department rules;

(2) To introduce any form of plant or animal life, except dogs when permitted by hunting rules of the department and service animals accompanying their masters;

(3) To remove, damage, or disturb any geological or paleontological features or substances;

(4) To remove, damage, or disturb any historic or prehistoric remains;

(5) To remove, damage, or disturb any notice, marker, or structure;

(6) To engage in any construction or improvement;

(7) To engage in any camping activity [that involves the erecting of a tent or other temporary structure] or to establish a temporary or permanent residence;

(8) To start or maintain a fire;

(9) To litter, or to deposit refuse or any other substance;

(10) To operate any motorized or unmotorized land vehicle or air conveyance of any shape or form in any area, including roads or trails, not designated for its use;

(11) To operate any motorized water vehicle of any shape or form in freshwater environments, including bogs, ponds, and streams, or marine waters, except as otherwise

provided in the boating rules of the [department of transportation,] department;

(12) To enter into, place any vessel or material in or on, or otherwise disturb a lake or pond;

(13) [No person shall] To engage in commercial activities of any kind in a natural area reserve without a written [special use]special-use permit from the board or its authorized representative[.];

(14) To have or possess the following tools, equipment, or implements: fishing gear or devices, including but not limited to any hook-and-line, rod, reel, spear, trap, net, crowbar, or other device that may be used for the taking, injuring, or killing of marine life; cutting or harvesting tools or gear, including but not limited to chainsaws, axes, loppers, any mechanized or manual sawtooth tool, seed pickers, or machete, that may be used for the taking, injuring, or killing of plant life; and hunting gear or tools that may be used for the taking, injuring, or killing of wildlife, except as permitted by the hunting rules of the department;

(15) To hike, conduct nature study, or conduct any activity with a group larger than ten in size;

(16) To be present in an area closed pursuant to section 13-209-4.5 or after visiting hours established pursuant to section 13-209-4.6;

(17) To anchor any motorized or nonmotorized water vehicle of any shape or form in marine waters;

(18) To enter into any cave, as defined in HRS section 6D-1, or any portion thereof;

(19) To conduct any other activity inconsistent with the purpose and intent of the natural area reserves system."

[Effective June 29, 1981] [Amended December 9, 2002] [Amended July 3, 2003] [Amended ___, 2005]

(Authority: HRS Sec. 195-5) (Implementation: HRS Sec. 195-5)

3. Section 13-209-4.5, Hawaii Administrative Rules, is adopted to read as follows:

"§ 13-209-4.5. Closing of areas.

The board or its authorized representative, with the approval of the commission, may close or restrict the public use of all or any portion of a natural area reserve for up to two years, when deemed necessary by the commission for the protection of the natural, geological, or cultural resources of the area or the safety and welfare of persons or property, by the posting of appropriate signs indicating the duration, extent, and scope of closure. Closures may be renewed with the approval of the board or its authorized representative and the commission. All persons shall observe and abide by the officially posted signs designating closed areas."

[Eff.] (Auth: HRS 195-5) (Imp: HRS 195-5)

4. Section 13-209-4.6, Hawaii Administrative Rules, is adopted to read as follows:

"**§ 13-209-4.6. Visiting Hours.**

The board or its authorized representative, with the approval of the commission, may establish a reasonable schedule of visiting hours for all or portions of a natural area reserve by the posting of appropriate signs indicating the hours during which the natural area reserve may be accessed. All persons shall observe and abide by the officially posted signs designating visiting hours."

[Eff.] (Auth: HRS 195-5) (Imp: HRS 195-5)

5. Section 13-209-5, Hawaii Administrative Rules, is amended to read as follows:

"**§ 13-209-5. Special-use permits.**

(a) The board or its authorized representative, with the approval of the commission or its authorized representative, may issue permits to conduct activities otherwise prohibited by section 13-209-4 for research, education, management, or for any other purpose consistent with chapter 195, Hawaii Revised Statutes.

[(b) The board or the commission may require a permit application to include an assessment of the potential environmental effect the special-use may have on the area concerned.]

(b) No permit may be valid for more than one year from date of issuance.

(c) All special-use permits shall be subject to standard conditions, as approved by the board, including but not limited to the following:

(1) The permittee shall adhere to project specifications given in the permit application;

(2) Disturbance of vegetation and wildlife shall be avoided as much as possible;

(3) Precautions shall be taken to prevent introductions of plants or animals not naturally present in the area. The permittee is responsible for making sure that participants' hiking clothes and equipment are free of seeds or dirt to lessen the chance of introducing any non-native plants or soil animals. Should an infestation develop, the permittee is responsible for eradication by methods specified by NARS - whether it occurs during or after the permit period

and even though it may be only indirectly attributable to the project activities;

(4) This permit is not transferable;

(5) This permit does not exempt the permittee from complying with any other applicable rule or statute;

(6) The State of Hawaii shall be released and held harmless from any and all liability for injuries or death, or damage or loss of property however occurring during any activity related to this permit.

(d) The board or its authorized representative may attach special conditions on the special-use permit, including but not limited to reporting requirements, limitations on the size of groups or the length of time for which the permit is valid. Failure to comply with any of these conditions shall render a permit void.

(e) All permittees shall carry the permit with them at all times while in the reserve and shall, upon request, show the permit to any law enforcement officer, the board or its authorized representative.

(f) Permits are not transferable. If the permittee is a partnership, joint venture, or corporation, the sale or transfer of 25% or more of ownership interest or stocks by dissolution, merger, or any other means, shall be deemed a transfer for purposes of this subsection and subject to the right of the department to terminate this permit effective the date of the sale or transfer.

(g) The board or its authorized representative may revoke or cancel a permit without prior notice when an emergency is declared by the Department of Land and Natural Resources or other proper authority or when the special-use poses an immediate threat to the health, safety, and welfare of the public or natural, geological, or cultural resources of the reserve.

(h) The board or its authorized representative may revoke or cancel any permit with thirty days written notice:

(1) for any infraction of the terms and conditions of the permit;

(2) upon a finding that the special-use damages or threatens to damage the integrity or condition of the natural, geological, or cultural resources in the reserve;

(3) upon a finding that the special-use poses a threat to the health, safety, or welfare of the general public or otherwise negatively impacts the general public's use and enjoyment of the reserve; or

(4) upon closure of a reserve pursuant to section 13-209-4.5.

[(c)](i) The provisions of this section shall not exempt the applicant from complying with any other applicable rule or statute."

[Effective June 29, 1981] [Amended ___, 2005]
(Authority: HRS Sec. 195-5) (Implementation: HRS Sec. 195-5)

6. Section 13-209-5.5, Hawaii Administrative Rules, is adopted to read as follows:

"§ 13-209-5.5. Applications for Special-use Permits.

(a) All applications for special-use permits shall be submitted in writing to the board or its authorized representative on the form prescribed by the department. The application shall contain the following information:

(1) name of applicant, and if relevant, affiliation and title;

(2) contact information, including name of primary contact, mailing address, phone number, and if available, email address;

(3) the period of time for which the permit is requested, not to exceed one year;

(4) the reserve(s) involved;

(5) a map illustrating the reserve and the location of the proposed special-use;

(6) a description of the proposed special-use;

(7) a discussion of how the proposed special-use satisfies subsections (b)(1) - (b)(6) below;

(8) an assessment of the potential environmental impact the special-use may have on the reserve or the surrounding area;

(9) signature of the applicant;

(10) an application fee of $50; and

(11) any other information as determined by the department.

(b) In evaluating the merits of an application for a special-use permit, the board or its authorized representative shall apply the following criteria:

(1) the proposed special-use cannot be conducted elsewhere;

(2) the proposed special-use is consistent with the purpose and objectives of the natural area reserve system;

(3) the proposed special-use is consistent with the management plan developed for the reserve;

(4) the proposed special-use provides a benefit (direct or indirect) to the natural area reserve system and/or to the individual reserve(s);

(5) the proposed special-use will not damage or threaten to damage the integrity or condition of the natural, geological, and/or cultural resources in the natural area reserve and adjacent area or region;

(6) the proposed special-use complies with provisions and guidelines contained in Chapter 205A, HRS, entitled "Coastal Zone Management," where applicable; and

(7) the applicant shall have complied with, or be in compliance with, the conditions of any previously approved permit;

(c) The applicant shall have the burden of demonstrating that the proposed special-use is consistent with the above criteria.

(d) The board or its authorized representative may hold a public hearing on an application where determined by the chairperson that the scope of the proposed special-use or the public interest requires a public hearing on the application. Notice of hearing shall be given not less than twenty days prior to the date set for the hearing. Notice of the time and place of the hearing shall be published at least once in a newspaper in the county where the natural area reserve is located;

(e) Any application for a special-use submitted to the board or its authorized representative pursuant to this chapter shall be reviewed by the department for completeness within sixty days from the date that the application was filed with the department. If it is found to be incomplete, the applicant shall be so notified by a letter stating the reasons therefor. If an application is accepted for processing, the applicant shall be notified by letter stating the commencement and completion dates for the processing of the application. If within one hundred and eighty days, or a time period as provided by law, after the department's acceptance of a completed application, the board or its authorized representative shall fail to render a decision thereon, the application for special-use permit shall be automatically approved with the standard conditions outlined in section 13-209-5(c), provided that the board may revoke this approval pursuant to subsections (g) and (h) of section 13-209-5. The one hundred and eighty day time period provided shall not commence until a completed application is accepted by the department. Physical receipt of an application by the department does not constitute acceptance. The one hundred and eighty day time period for decision may be extended for another one hundred and eighty days at the request of the applicant to give the board additional time to review and make a decision on the application."

[Eff.] (Auth: HRS 195-5; 91-13.5) (Imp: HRS 195-5; 91-13.5)

7. Material, except source notes, to be repealed is bracketed. New material is underscored.

8. Additions to update source notes to reflect these

amendments are not underscored.

9. These amendments to chapter 13-209, Hawaii Administrative Rules, shall take effect ten days after filing with the Office of the Lieutenant Governor.

APPROVED FOR PUBLIC HEARING:

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Deputy Attorney General

State of Hawaii
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Forestry and Wildlife
Honolulu, Hawaii 96813

January 13, 2006

Chairperson and Members
Board of Land and Natural Resources
State of Hawaii
Honolulu, Hawaii

Land Board Members:

SUBJECT: REQUEST FOR APPROVAL TO ENTER INTO EIGHTEEN CONTRACTS TO IMPLEMENT THE HAWAII INVASIVE SPECIES COUNCIL RESEARCH AND TECHNOLOGY GRANT PROGRAM PROJECTS: WITH THE BISHOP MUSEUM FOR "IMPLEMENTING EARLY DETECTION;" WITH THE UNIVERSITY OF HAWAII FOR A STUDY OF "DINOFLAGELLATES IN BALLAST WATER;" WITH THE UNIVERSITY OF HAWAII FOR A STUDY OF "INVASIVE ANT CONTROL;" WITH THE USDA NATIONAL WILDLIFE RESEARCH CENTER FOR DETERMINING "RODENTICIDE EFFICACY;" WITH THE USDA NATIONAL WILDLIFE RESEARCH CENTER TO RESEARCH A "BROWN TREE SNAKE ATTRACTANT;" WITH THE UNIVERSITY OF HAWAII FOR FINDING "*ERYTHRINA* GALL WASP PARASITOIDS;" WITH THE USDA NATIONAL WILDLIFE RESEARCH CENTER FOR TESTING "BROWN TREE SNAKE BAITS;" WITH THE UNIVERSITY OF HAWAII FOR "INVASIVE SPECIES DATABASE ENHANCEMENTS;" WITH THE HAWAII DEPARTMENT OF AGRICULTURE FOR "MANAGEMENT OF *ERYTHRINA* GALL WASP;" WITH THE USDA AGRICULTURAL RESEARCH SERVICE FOR "NETTLE CATERPILLAR LURE APPLICATIONS;" WITH THE UNIVERSITY OF HAWAII OR TRI-ISLE RESOURCE CONSERVATION AND DEVELOPMENT FOR A "COQUI-FREE CERTIFICATION PROGRAM;" WITH BISHOP MUSEUM FOR "AQUATIC INVASIVE SPECIES EXPERTISE;" WITH MCCLAY ECOSCIENCE FOR A "*MICONIA* BIOCONTROL SURVEY IN MEXICO;" WITH THE USDA INSTITUTE OF PACIFIC ISLANDS FORESTRY OR

THE UNIVERSITY OF HAWAII TO SURVEY FOR A "HIMALAYAN BLACKBERRY BIOCONTROL;" WITH THE USDA INSTITUTE OF PACIFIC ISLANDS FORESTRY OR THE UNIVERSITY OF HAWAII FOR A "*MICONIA* BIOCONTROL EVALUATION;" WITH COLORADO STATE UNIVERSITY TO STUDY "WEST NILE VIRUS IN PARAKEETS;" AND WITH THE US FISH AND WILDLIFE SERVICE OR THE NATIONAL WILDLIFE RESEARCH CENTER TO REFINE A "MULTI-PEST EXCLUSION FENCE"

This board submittal approves contractual relationships for eighteen projects that address research and technology needs for invasive species priorities, and authorizes the Chairperson to develop and enter into contracts for these services, subject to the availability of funds and approval as to form by the Attorney General's Office.

BACKGROUND:

The 2003 State Legislature authorized the creation of the Hawaii Invasive Species Council, and stated "the silent invasion of Hawaii by alien invasive species is the single greatest threat to Hawaii's economy, natural environment, and the health and lifestyle of Hawaii's people and visitors." Hawaii is one of the seven states in the nation that has recognized the need for coordination among all state agencies, at a cabinet level, that have responsibility to control invasive species on the ground, as well as regulate the pathways in which invasive species can gain access into the state.

The creation of the council (whose members are the directors or chairs of the Departments of Land and Natural Resources (DLNR,) Agriculture (DOA,) Business, Economic Development, and Tourism (DBEDT,) Health (DOH,) Transportation (DOT) and University of Hawaii (UH,) and other department directors (Hawaiian Home Lands (DHHL,) Commerce and Consumer Affairs (DCCA) and Defense (DOD)) now provides the institutional framework for leadership and coordination for a statewide invasive species prevention and control program.

The first official meeting of the HISC convened on October 29, 2003. HISC members adopted a working committee structure to look at laws, policies, procedures, and needs in the areas of ongoing prevention, early detection/rapid response, research and the application of new technology, and increasing public awareness.

HISC, through the DLNR, received an administrative budget of $4 million for the year to provide support for the operations of the HISC and its cooperating partners, to develop a comprehensive state-wide invasive species prevention, control, research, and outreach program. These funds will be matched 1:1 with non-state dollars. Of that amount, $700,000 was budgeted for the Research and Technology Program. A total of fifty sealed project proposals were received and eighteen were selected for funding.

These eighteen Research and Technology projects total $600,000 and are matched with $711,043 in non-state dollars. They are:

Attach-ment	Project	Recipient	Amount
A	Implementing Early Detection	Bishop Museum	$80,000
B	Dinoflagellates in Ballast Water	University of Hawaii (UH)	$71,000
C	Invasive Ant Control	University of Hawaii	$70,000
D	Rodenticide Efficacy	USDA NWRC	$69,700
E	Brown Tree Snake Attractant	USDA NWRC	$60,835
F	*Erythrina* Gall Wasp Parasitoids	University of Hawaii	$45,000
G	Brown Tree Snake Baits	USDA NWRC	$30,725
H	Invasive Species Database Enhancements	University of Hawaii	$29,288
I	Natural Enemies of *Erythrina* Gall Wasp	HDOA	$23,899
J	Management of *Erythrina* Gall Wasp	University of Hawaii	$22,000
K	Nettle Caterpillar Lure Applications	USDA Ag. Research Svc.	$20,000
L	"Coqui-free" Certification Program	Tri-Isle RCD or UH	$15,000
M	Aquatic Invasive Species Expertise	Bishop Museum	$15,000
N	*Miconia* Biocontrol Survey in Mexico	McClay Ecoscience	$14,625
O	Himalayan Blackberry Biocontrol	Inst. Pac. Is. Forestry. or UH	$12,000
P	*Miconia* Biocontrol Evaluation	Inst. Pac. Is. Forestry. or UH	$7,700
Q	West Nile Virus in Parakeets	Colorado State University	$6,864
R	Multi-pest Exclusion Fence	USFWS or USDA NWRC	$6,364

The project with Hawaii Department of Agriculture (Attachment I) may be awarded as a cooperative agreement with an interdepartmental transfer. Nine other projects of amounts less than $25,000 (Attachments J-R) may be fulfilled with purchase orders instead of contracts. The project Multi-pest Exclusion Fence (Attachment R) may be combined with the project Rodenticide Efficacy (Attachment D) to create one contract for both projects with the USDA NWRC as the recipient of an amount of $76,064.

CONTRACT PROVISIONS

The invitation for bids for these proposals was published on the State Procurement Office web site on October 3, 2005 (IFB No. HISC RT 004) and November 7, 2005 (IFB No. HISC RT 005).

Contracts will be negotiated with the principal investigators authorized by the recipients, to implement the projects according to their project descriptions. The standard state contract form will be used and approved by the Attorney General's Office.

Upon approval by the board, the division will submit the contracts for review and approval as to form by the Attorney General, and process the documents for signature by the chairperson.

RECOMMENDATION:

That the board: 1) approve implementation of the HISC Research and Technology Program for FY06; and 2) authorize the chairperson to negotiate and execute contracts subject to:

a. Scope of services (contract deliverables) as described in the attached proposals,
b. Availability of state funds; and
c. Approval as to form by the Attorney General's Office.

Respectfully submitted,

PAUL J. CONRY, Administrator
Division of Forestry and Wildlife

Attachments A-R

APPROVED FOR SUBMITTAL:

for PETER T. YOUNG, Chairperson
Board of Land and Natural Resources

NEW PROPOSAL

To

HAWAII INVASIVE SPECIES COUNCIL –
RESEARCH AND TECHNOLOGY PROGRAM

From

BISHOP MUSEUM
1525 Bernice Street
Honolulu, Hawaii 96817

IMPLEMENTING EARLY DETECTION IN HAWAII

1 January 2006 – 31 March 2007

$98,946

n Y. Brown
ent, CEO

Allen Allison
Vice President, Science

Imada
Specialist, Botany

Problem Statement

Invasive species place an immense strain on the environment, economy and general welfare in nearly every country on earth. Recent estimates have placed the cost of damage and loss due to invasive species at over $137 billion in the United States alone (Pimental et al. 2001). Hawaii's unique isolation makes it particularly susceptible to biological invasions (Loope and Mueller-Dombois 1989). It is clear that that many aggressive plant and animal species not yet established in Hawaii can, if introduced, permanently alter the unique landscape of Hawaii leading to species extinction, environmental degradation, and enormous economic strain.

With an estimated 20–50 new species becoming established in Hawaii every year (Loope and Canfield 2000), an effective system of prevention and early detection is imperative to safeguarding Hawaii. The island based Invasive Species Committees (ISCs) have proven to be an effective mechanism of rapid response and the control of incipient pest species. However, the ISCs lack a comprehensive early detection program for ensuring the greatest probability of detecting new invasive species.

There have been several successful early detection efforts in Hawaii, but they stand as isolated island-specific projects. This proposal will build upon previous detection work and capitalize on local and foreign expertise and experience to develop and subsequently implement a comprehensive detection framework for Hawaii. This model will incorporate the need to systematically re-evaluate detection strategies and re-survey identified pathways and high risk sites in order to achieve continual comprehensive early detection of our islands. This project will then tailor the statewide detection model to the island of O'ahu, where we will implement a three-year on the ground detection program. In addition, this proposal will begin a program of efficient, non-native species identification and cataloguing at the Bishop Museum.

Methodology:

This is a proposal to fund the first year of a three year project to develop and implement a statewide program of comprehensive early detection, and for its initial implementation on O'ahu. This proposal is a pilot project that will enable the ISCs and the Bishop Museum to forge a long term partnership to jointly ensure stable funding for the early detection of and rapid response to invasive species in Hawaii. There are three main goals of this project:

- Detection Plan Model: Using previous research and experience, this project will develop a written Detection Plan Model that will help all ISCs pinpoint new invasive species before they become established beyond hope of eradication. Each island will be able to tailor the model to suit their needs. This proposal requests funding to contract botanists Forest and Kim Starr, to lend their detection expertise in developing the plan.
- Rapid ID Program: New potentially invasive non-native plant species found throughout the state will be rapidly identified and processed to assist with detection efforts. This proposal includes funding for two positions that would be housed at the Bishop Museum; one botanist and one technician (hereinafter referred to as the detection staff). These positions will become a stable taxonomic resource that all invasive species groups can use to perform early detection surveys and coordinate statewide Rapid ID. In addition to helping develop the detection plan, Forest and Kim Starr will help train the detection staff.
- O'ahu Early Detection Program: OISC will modify the model to suit O'ahu and implement it utilizing the detection staff to perform the detection surveys. Data from implementation will be used to further refine the plan

Detection Plan Model

The first part of this project will build on previous detection efforts in Hawaii and elsewhere and establish a model that all the ISCs HDOA and partner agencies can use to find and eradicate introductions of potentially invasive species in Hawaii. This will be a model that can be used statewide but tailored for local implementation on each island. This work will entail collaboration with researchers ,managers and contributing agencies across the state. The primary questions that will be addressed in this plan are:

- **How can the ISCs make early detection of new invasive species comprehensive and systematic?** Previous early detection surveys have been limited to one-time surveys and eradication. The detection plan model will identify and assess the pathways and high-risk sites for incipient invasive species to enter an island and provide guidance on the number of times these areas should be surveyed and the methodology assessed so that a species cannot become established. Forest and Kim Starr will serve an integral role in compiling and reviewing previous detection models to establish a statewide detection model.
- **When does a species merit control?** Since limited resources are available to thwart the greatest threats, the plan will develop criteria to help resource managers decide when rapid response is necessary. The plan will: define the level of establishment of a given species that merits rapid response, criteria to assess the level of establishment of given species on an island, and outline the protocol for rapid response and eventual eradication for a given species.
- **How do you achieve comprehensive detection on O'ahu?** Once the statewide plan is established, OISC, with help from invasive species experts, will identify the highest risk areas on O'ahu. OISC field staff will prioritize these areas and create a timetable to repeatedly survey the highest risk sites.
- **How can we combine already existing data into one statewide, early detection database?** The USGS BRD Pacific Basin Information Node (PBIN) is working to build the technical infrastructure to support statewide early detection efforts. They are in the process of developing a pilot multi-agency early detection reporting system for Maui. This system could potentially be implemented throughout the state. In addition, PBIN is submitting a HISC proposal to enhance the current database systems of four of the five ISCs to accommodate local efforts. PBIN will play a vital role in ensuring that the databases and data collection and reporting methodologies meet statewide standards and facilitate the integration of early detection data into one statewide system.

O'ahu Early Detection Program

The O'ahu Early Detection Program will test and implement the Detection Plan Model on O'ahu. As comprehensive early detection is a daunting task, this project will spread out early detection on O'ahu over a three-year period

Initial work to develop a survey methodology for year one of the O'ahu Early Detection program will revolve around building upon previous surveys and work preformed, to develop a survey methodology for year one of the O'ahu Early Detection program. Forest and Kim Starr, OISC, HDOA and local input from the detection community will be instrumental in developing this program. Once the program is established, the detection staff will perform the on the ground surveys with the GIS and field support of OISC. The detection staff will utilize the Detection Plan Model and O'ahu Early Detection plans to assess and evaluate their findings to prioritize species for control. OISC will then coordinate the rapid response and eradication of the given

species. OISC in conjunction with appropriate partners, such as HDOA and DLNR, will also focus on targeted searches and assessments of high risk sites and commercial plant pathways into the island. These areas include:

- Botanical gardens
- Nurseries
- High risk sites: Agriculture research centers, and other potential sites that have not yet been identified
- Pathways: Swap meet, local markets, and other 'unregulated' pathways that have not been identified

Education/Outreach: OISC will increase public awareness of the importance of early detection through community outreach work trips and public presentations. This will hopefully increase reporting

Rapid ID Program

The Botany unit of the Department of Natural Sciences at the Bishop Museum is the primary repository for plant vouchers in the State, and Botany staff annually provide identifications for hundreds of specimens at the request of a variety of "clients," including the State Department of Agriculture; resource management staff for federal, state, and private organizations; and the general public. Due to resource constraints, however there can often be a notable time lag in identification and cataloguing of specimens submitted to the Museum, jeopardizing detection and control efforts that depend on rapid identification of species. We believe there is an urgent need to supplement Bishop Botany staff with additional hired staff dedicated to facilitating and coordinating the timely processing, identification, and cataloguing of potentially invasive species to be vouchered as part of this project.

The Bishop Museum and OISC propose to develop a statewide standardized methodology for species acquisition, identification, and cataloguing in the Museum. The ISCs will work directly with the detection staff to ensure rapid identification and cataloguing of species for early detection, in addition to ensuring the vouchers are correctly tracked in a database. The detection staff will serve as the coordinating entity at the Museum to track, voucher, and ensure efficient identification and information turnaround to the ISCs for statewide identification and detection of species.

In addition, PBIN will work with the Rapid ID program to ensure that all information collected an tracked will adhere to statewide data standards and protocols for eventual use in a statewide database for tracking and assessing species establishment in Hawaii.

Schedule of Activities:

January - February 2006:

Develop and submit a position description for the detection positions (OISC, Bishop Museum)

January - March 2006:

Establish methodology for targeted detection survey for year one of O'ahu Early Detection Program: (Bishop Museum, Forest and Kim Starr and OISC

Establish data field collection and recording requirements to a statewide standard: Bishop Museum, Forest and Kim Starr, and PBIN)

Develop statewide methodology for processing and tracking non-indigenous species vouchers to implement the Rapid ID Program: (Bishop Museum, Forest and Kim Starr and PBIN)

February - April 2006:

Recruit and hire positions (OISC and Bishop Museum)

April 2006 - March 2007:

Perform year one of the O'ahu Early Detection Program (detection staff)

March - April 2006

Train new staff and implement Rapid ID program at the Bishop Museum. This includes educating partner ISCs and the Hawaii detection community regarding the protocols for species vouchering and submission protocols.(OISC and Bishop Museum)

February - July 2006:

Develop a draft Detection Plan Model: (Invasive Species Committees, Forest and Kim Starr, the detection staff, Bishop Museum, PBIN and the Hawaii detection community)

March - September 2006:

Develop draft plan for year 2 and 3 of the O'ahu Early Detection Program (OISC and the Hawaii detection community)

September - December 2006

Refine year 2 and 3 of plan for O'ahu Early Detection Program (OISC and the Hawaii detection community)

Begin testing year 2 and 3 survey models and techniques (OISC and the Hawaii detection community)

February 2007:

Provide HISC with: Annual report of the O'ahu Early Detection Program, the Rapid ID program and the Detection Plan Model. (OISC, Bishop Museum)

Description of deliverable products:

- Detection Plan Model: A formal document outlining the steps an ISC should take for developing the capacity to rapidly respond to new introductions. Both the statewide model and the refined plan for the O'ahu Early Detection Program will be submitted by the end of year one of the project.
- Rapid ID Program Report: A summary of the number of species that have been identified and catalogued in both the actual and virtual collections. A breakdown of resources and hours—those funded by this proposal and both those contributed from other sources—will be included.
- O'ahu Early Detection Program Annual Report: Annual report summarizing O'ahu survey findings, including a summary of species prioritized for control, and any subsequent control or survey work. This will include a breakout of time and resources utilized on this aspect of the project, including contributed hours. Education and outreach activities associated with the O'ahu Early Detection Program will be included in the annual report.

Personal and Partners:

Clyde Imada is a botanist (Research Specialist) who has worked in the Botany unit of Bishop Museum for over 20 years. Clyde will serve as the principal investigator, and will co-manage the project with *Ryan Smith*. Clyde and *Napua Harbottle*, the Collections Manager of Botany, will head the development of the Rapid ID Program, train and oversee the detection staff, and assist with the development of the Detection Plan Model. In addition Clyde, Napua,

and other Bishop Museum staff will be assisting with voucher identification and ensuring proper cataloguing of specimens.

Ryan Smith is the coordinator of the O'ahu Invasive Species Committee. He will co-manage the project with *Clyde Imada.* Ryan will be responsible for coordinating with the various partners and resources to ensure project success. He and OISC will play an integral role in coordinating and overseeing the development of all the protocols and plans for this proposal. In addition, OISC will be the primary entity coordinating, tracking and performing any control/eradication work on new incipient target species that may arise from the O'ahu Early Detection Program. Finally, OISC staff will work as needed to train and assist the detection staff to perform detection surveys.

Forest and Kim Starr are taxonomists based on Maui that have led early detection surveys on Maui and have vast experience performing botanical and detection surveys throughout the state. They will be contracted to assist in the development of all the project's protocols and plans. They will provide in-kind services to teach the detection staff their survey and detection methodology, and to provide field and species identification assistance as the staff implements the O'ahu Detection Plan.

Pacific Basin Information Node (PBIN). PBIN strives to enable effective biodiversity management in the Pacific Basin by providing the framework and infrastructure for biodiversity information acquisition, analysis and application. PBIN will ensure that data generated from early detection efforts meet standards and protocols for the ISCs statewide reporting system and can be aggregated in a comprehensive early detection and rapid response database for the state of Hawaii. In addition, PBIN will provide in kind-services as a data consulting through the development and implementation of all the proposed projects. PBIN is submitting a proposal to build off existing databases and reporting created for the ISCs, make enhancements and accommodate early detection of vertebrates and invertebrates. .

Hawaii detection community (HDC). This represents a diverse collaboration of resource managers and scientists who have historically been involved in early detection strategies and field programs in Hawaii. This community will provide integral counsel and input to the development of the Detection Plan Model and the O'ahu Early Detection Program. Individuals and organizations include *Neil Reimer (HDOA), Anne Marie LaRosa (USFS),* Kealii Bio *(USBRD),Teya Penniman (MISC), Keren Gunderson (KISC), Julie Leialoha (BISC), Lori Buchanan(MoMISC), Julie Denslow (Institute of Pacific Islands Forestry), Curt Daehler (UH faculty / Weed risk assessment), Carter Smith (CGAPS),Rob Cowrie* (USDA), *Lloyd Loope* (USGS), Aphis/USDA staff, in addition to others not identified here.

Budget:

Item	Requested	Match and In-kind
1. Staff: • **Botanist: $48,913** • **Technician: $24,797**	**$73,709**	**Recruitment, training and identification assistance: $26,253**
2. Materials and supplies $5000	**$4,500**	**Materials and Supplies = $25,400**
3. Development of detection plans and project support • **Contracting Forest and Kim Starr $6,000**	**$6,000**	**Development of detection plans and project support $17,191**
4. Grant Administration (17.5%)	**$14,737**	**Bishop Museum absorbed overhead: $35,997**
TOTAL	**$98,946**	**$104,841**

Are Toxic Dinoflagellates found in Ballast Water of Ships Visiting Hawaii?

A Funding Proposal for the Hawaii Invasive Species Council Research and Technology Grant 2006 requesting the amount of $71,000.00

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ATTACHMENT B

The Hawaii Department of Land and Natural Resources/Division of Aquatic Resources and Dr. Ruth Gates of the Hawaii Institute of Marine Biology request funding for a project to develop baseline data for managing potentially harmful invasive algal species.

PROBLEM STATEMENT

A harmful algal bloom (HAB) is defined as a bloom that has deleterious effects on plants, animals and/or humans. HABs, such as red tides, have been occurring for centuries. In the recent years, they appear to be more frequent and extensive, both in the United States and worldwide (Epstein,1998; Fleming and Baden 1998; Hallagraeff,1993). Their massive economic impacts have been documented in many studies. In 1997, the dinoflagellate *Pfiesteria piscicida* caused a massive fish kill in the Chesapeake Bay that resulted in approximately $40 million dollars in losses to the fishing industry. *Pfiesteria piscicida* and a second species, *Pfiesteria shumwayae*, have been implicated as the primary causative agents of well over a billion fish deaths in North Carolina (NC) estuaries by the local Centre for Applied Aquatic Ecology (CAAE). HAB impacts can occur when marine fauna are killed by microalgal species that release toxins and other compounds into the water or that kill without toxins by physically damaging gills or by creating low oxygen conditions as bloom biomass decays. These impacts frequently occur at aquaculture sites where caged fish cannot escape the harmful blooms. Farmed fish mortalities from HABs have increased considerably in recent years, and are now a major concern to fish farmers and their insurance companies. It has also been recognized that there can be impacts from toxic blooms in virtually all compartments of the marine food-web due to adverse effects on viability, growth, fecundity, and recruitment (Smayda 1992). However, HABs can affect more than fish populations and the fishing industry. Human exposure to water infested by *Pfiesteria piscicida* cells has resulted in blisters and abscyses forming on exposed skin. According to researchers at the NC State University, other symptoms of *Pfiesteria piscicida* exposure are a "drugged" effect; red eyes, severe headaches, blurred vision, nausea/vomiting, asthma-like difficulty breathing, kidney and liver dysfunction, acute short-term memory loss, and serious difficulty being able to read, remember one's own name, dial a telephone number, or do simple arithmetic. Harmful health effects and even death resulted from humans consuming shellfish contaminated by HABs. In addition, marine mammals have also been shown to be affected by HABs. In 1996, at least 149 manatees (Trichechus manatus latirostris) died in a large epizootic along the southwest coast of Florida. At about the same time, a bloom of the brevetoxin-producing dinoflagellates, *Gymnodinium breve* was in the area, and laboratory results implicated the *G. breve* as the cause of death. (Bossart et al., 1998.)

The transport of these organisms through ballast water has been well documented. Ruiz et al. (2000) measured levels of bacteria, virus-like particles (to mimic viruses which are too difficult to detect), and the bacteria *Vibrio cholerae* (which causes human cholera) in the ballast tanks of vessels entering Chesapeake Bay ports from foreign carriers, and found very high numbers. Their data indicated that viable cell populations of *V. cholerae* can be frequently delivered to estuaries by ships coming from foreign ports. They concluded that coastal ecosystems are frequently invaded by microorganisms from ballast water, including species that can induce resting stages, in which they lay dormant until the new environment has the right physical conditions for rapid growth. Dinoflagellate cysts present in discharged ballast sediments are believed to be responsible for the introduction of Paralytic Shellfish Poisoning-causing algal

blooms which cause and the subsequent disruption of the shellfish culture industry in Tasmania (Kelly, 1993).

The introduction of invasive dinoflagellates into Hawaii by ballast water discharge is highly possible given the historical evidence of introductions in this manner in other parts of the world. This is compounded by the fact that currently proposed ballast water regulations would only produce an effective discharge of 95% of potentially infected ballast water, leaving 5% as an entry-point for non-native organisms. Complicating the interpretation of dinoflagellate invasions via ballast water is the fact that there is almost nothing known about the natural populations of dinoflagellates and other such microorganisms in Hawaiian waters. Without this baseline, it will be impossible to design a management strategy aimed at identifying and ameliorating invasions of dinoflagellate in ballast water. In addition, the relationship between corals and their symbiotic zooxanthellae (which are also dinoflagellates) is poorly understood, as are the exact mechanisms involved in coral bleaching and reacquisition of available symbionts from the environment after a bleaching event. The introduction of large numbers of non-native or invasive dinoflagellates has implications in terms of the delicately balanced native communities (including coral symbionts) and thus ramifications for the future of Hawaii's valuable coral reef resources.

The Division of Aquatic Resources, Hawaii will work with researchers at the Hawaii Institute of Marine Biology in order to produce an effective baseline of native and previously introduced dinoflagellate communities in Hawaiian waters. This type of collaboration is necessary to establish a scientific basis for future management regulations of new species introductions and will benefit both scientific and management sectors.

METHODOLOGY

Water samples (2 liter volumes) will be collected from 3 locations:

1. Ballast tanks from ships in Honolulu Harbor. The ballast tanks of 4 tankers originating from each of three embarkation points (n=4 per embarkation point)
2. Honolulu Harbor water (n = 4)
3. A quarter mile outside Honolulu harbor (n = 4)
4. A site in the Northwest Hawaiian Islands (n = 4)

Each sample will be divided in two and processed independently to examine the reproducibility within the samples. The dinoflagellates in the water samples will be deposited onto filters and a total genomic DNA extracted and amplified using PCR (polymerase chain reaction) to target the large subunit rDNA (28s) region of the nuclear ribosomal array that identifies dinoflagellates to family and genus, The PCR products will be cloned to separate 28S sequences representing different dinoflagellates in the seawater samples and the clones characterized by sequencing. To identify which dinoflagellates are present, the sequences obtained will be compared to all known dinoflagellate sequences in GenBank, a world-wide library of genetic information.
All samples will be processed in a similar way and the data analyzed to address the following questions:

1. Are there species of dinoflagellates in ballast water that are not found in Hawaiian waters?
2. If so, are any of these species of dinoflagellates known to produce toxins or coral symbionts?

3. Do the ballast water samples taken from ships originating from specific locations have dinoflagellate communities that are distinct from one another?

SCHEDULE OF ACTIVITIES

Months 1 - 3:

Identify embarkation points for ships coming to Honolulu harbor; obtain permissions to sample ballast water; obtain ballast water samples, harbor samples and outside harbor samples; isolate genomic DNAs and store at –80oC.

Months 4 - 9

PCR and cloning of stored genomic DNAs; Obtain and process NWHI water samples; Sequence clones.

Months 10-12

Taxonomic identification of sequences and analysis of dinoflagellates diversity in the different water samples; Report generated; results presented at a UH seminar and development of publication for peer reviewed journal.

BUDGET

Salary plus fringe	Technician	$55,000
Materials and supplies		$16,000
Total HISC Funds Requested		**$71,000**

We are requesting a full time MS level technical position to complete this labor and technically intensive endeavor. The individual will be hired through RCUH and the work completed in Ruth Gates's fully equipped molecular laboratory at HIMB.

The materials and supplies for this project reflect the cost of PCR and cloning reagents (e.g. a cloning kit for 40 samples costs $638.00 from invitrogen) and sequencing at $4 per sequence – note this is reduced from the normal cost of $6/sequnce as Gates contributed funds to purchase the equipment. We anticipate generating between 2500 and 4,000 sequences on this project.

Non-State of Hawaii Matching Funds

Post doc in Gates Lab to collect NWHI samples and supervise technician (FTE 0.25)	$15,000
NOAA funded NWHI Cruise costs (<5% cost)	$30,000
AIS coordinator (USFWS funded salary plus fringe (10%)	$ 5,200
Sequencing discount $2/sequence	$ 8,000
Core Facility Manager (NSF EPSCoR FTE .15)	$ 7,800
Sequencing of 1500 template at $4 per sequence Gates lab federal funds	$ 6,000
Total Non-State of Hawaii Matching Funds	**$72,000**

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Title: Developing New Techniques for Invasive Ant Control in Hawaii

Principle Investigator: Paul D. Krushelnycky

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Funding amount requested: $80,000 over 2 years

Problem Statement

Invasive ants are among the most damaging of Hawaii's invasive species. There are no native ants in Hawaii, yet in the past several hundred years over 40 ant species have been introduced to the state. Some of these species have caused substantial impacts to native Hawaiian biodiversity, and are pests of agriculture and urban areas. In addition, recent and potential introductions, such as the little fire ant and red imported fire ant, respectively, have the ability to exert strong impacts on tourism and other sectors of the economy (Krushelnycky et al. 2005a).

As with most invasive species, prevention and improved quarantine procedures are the most effective means for handling new and expanding ant species. Nevertheless, techniques for controlling and even eradicating existing populations of the state's most invasive ant species are critical for situations of rapid response to incipient incursions, as well as situations in which biodiversity and other interests can be protected by removing well-established but localized ant populations. While eradication of well-established insects is usually unlikely to succeed, invasive ants represent a special case in which biological traits related to their invasiveness also provide a critical weakness that can be exploited: most of the truly damaging invasive ant species exhibit a unicolonial social structure in which mating flights do not occur, new queens bud from existing nests and disperse short distances by walking, and populations can therefore exist as discrete, localized entities even when the species' distribution is much wider. Eradication of local populations can thus result in the permanent removal of these species from particular areas of concern, as long as re-introduction by humans can be prevented or quickly detected.

Efforts to eradicate invasive ant populations for conservation purposes have in fact gained considerable momentum in the last decade. A growing number of successes around the Pacific provide evidence that under the right circumstances, ant eradication is possible (Krushelnycky et al. 2005a). All of these efforts have involved the use of attractive baits formulated with insecticidal toxicants, but the formulations and methods of application have varied considerably. Different species respond to different baits, and different situations call for different active ingredients and methods of application. Developing multiple management tools for invasive ants will greatly improve the state's ability to address these problem species.

At Haleakala National Park (HALE), the Argentine ant, *Linepithema humile* (Mayr), has emerged as one of the most important threats to subalpine shrubland and alpine zone arthropods, which constitute the majority of native biodiversity in these areas. Since at least 1967, the Argentine ant has been slowly but steadily spreading within the park, with two discrete populations now covering over 625 ha. Numerous experiments have been conducted over the past ten years at HALE in an attempt to develop a method for eradicating the Argentine ant using a variety of commercial and experimental pesticidal ant baits (e.g. Krushelnycky and Reimer 1998a,b). While some of these have been very effective in reducing numbers of ants, none has been able to eliminate all nests in experimental plots. Research into a secondary management goal of ant population containment was initiated in 1996. By treating only expanding margins of the park's two ant populations with an ant pesticide, rates of outward spread have been reduced by over 60%. This strategy was implemented from 1997 to 2004, and was regarded as worthwhile, but it was discontinued after 2004 because of changes in the pesticide formulation and resultant lower efficacy (Krushelnycky et al. 2005b).

At this point in time we are renewing research into alternate control techniques for the Argentine ant at HALE, and have begun investigating the toxicant boric acid. Boric acid has been used to control ants for decades, however recent laboratory and field studies suggest that when formulated at very low concentrations in sugar water baits, boric acid is more effective against a variety of invasive and "tramp" ant species, including the Argentine ant (e.g. Hooper-Bui and Rust 2000). A 50 m by 50 m experimental plot using 0.5% boric acid bait stations at HALE produced promising results in 2004-2005 (Krushelnycky et al. 2005b). Even though eradication was not achieved, nearly all nests were killed and the boric acid bait performed equal to or better than a variety of previously tested baits. We feel that further experimentation with boric acid will yield better results, possibly including eradication from test plots. Specifically, we predict that a 1% boric acid formulation will work more quickly and effectively, and because boric acid acts much more slowly than most insecticides, a larger experimental plot size should prevent re-invasion and provide more reliable results.

Because the two ant populations at HALE are so large, we hold the view that any successful eradication strategy may have to work incrementally from the periphery of the populations to their centers. If a successful eradication technique can be developed, this periphery-to-center approach would focus resources on smaller border areas at any one time and should therefore increase the chances of success. Further outward spread of the ant populations would concurrently be prevented.

We propose to test the commercial product Drax Liquidator Ant Bait, a sugar-based liquid bait formulated with 1% boric acid, in large population border plots at HALE. These plots will simultaneously evaluate the effectiveness of this bait for Argentine ant population containment and eventual eradication. We will test different bait station spacing intervals, since effort and cost greatly decrease as bait station interval increases.

Liquid baits, deployed in bait stations, are necessarily more laborious and costly than granular baits, which can be applied more quickly and easily with hand spreaders, or even aerially. However sugar water-based boric acid baits have certain advantages over granular products. Sugar water is highly attractive to a wide variety of invasive ant species, and while some granular products work well for some ant species, other invasive ant species, including the Argentine ant, have proved to be insufficiently responsive to commercial granular baits. Liquid baits are more easily retrieved and shared among nestmates than granular baits, and their continued presence in bait stations provides longer and more thorough access for the target ants (while limiting access for non-target species). Finally, boric acid itself is one of the most benign toxicants available, making it more suitable for use in highly sensitive situations.

The commercial product Drax Liquidator Ant Bait is not currently registered for the use pattern we propose. Consultation with the Hawaii Department of Agriculture, however, confirmed that we should have no difficulty in obtaining an Experimental Use Permit for this experiment (L. Kobashigawa, pers. comm.). If this bait formulation proves to be effective, we will design and conduct the experiments necessary to apply for a Special Local Need label extension from the U.S. E.P.A. A similar extension was obtained for bait station use of Arndro granular ant bait in Hawaiian fruit orchards (Taniguchi et al. 2003).

While this experiment specifically targets Argentine ants at HALE, we stress that the technique we propose to investigate, if successful, could eventually have much wider application in Hawaii. First, Argentine ants also occur in middle to high elevation natural areas on Kauai and Hawaii Island, including both state and federal land. Some of these populations are relatively restricted in size and could be targeted for eradication. Second, as mentioned, sugar water is attractive to many invasive ant species. Boric acid bait stations could be an important tool to replace or supplement granular baits in sensitive situations, such as in agricultural parcels or next to water courses. The inability to broadcast Amdro granular ant bait in orchards, for example, was an important obstacle in early efforts to eradicate the little fire ant on Hawaii Island. We have been told that boric acid is even compatible with organic farming (J. Klotz, pers. comm.). These advantages could become extremely important should the little fire ant spread to new islands, or if the red imported fire ant were to establish in the state. The successful development of boric acid bait stations for non-urban settings could therefore greatly strengthen the array of tools available to address invasive ants, under various conditions, in Hawaii.

Methods

The regulations of Hawaii's Experimental Use Permit (EUP) provision limit total area of the experiment to 10 acres. Because of these limitations, we will initially establish two treatment plots and one control plot on an advancing boundary of HALE's lower Argentine ant population. The two treatment plots will test two bait station spacing intervals (10 m grid and 20 m grid), and will be 140 m wide by 120 m deep. They will be positioned such that the plots will extend roughly 20 m beyond the ant population boundary, and therefore the plots will encroach approximately 100 m into the population (for a length of 140 m). The adjacent control plot will be separated from the nearest treatment plot(s) by at least 60 m, and will have dimensions equal to the central monitoring core of each of the two treatment plots: 60 m wide by 80 m deep. A 40 m buffer provisioned with boric acid bait stations will therefore surround the central monitoring core in the two treatment plots and should prevent reinvasion from outside the plots.

Monitoring in the treatment and control plots will use methods developed over a decade at HALE (Krushelnycky *et al.* 2005b), and will include monthly bait card monitoring and nest surveying. We will use a tuna/corn syrup mix on monitoring bait cards spaced every 20 m in the monitoring core. These bait cards attract foraging workers and provide a relative estimate of population levels over the treatment period. Bait cards will also be used on three transects extending out from the population borders in each plot; these will be used to track rate of outward population spread in both treated and control plots. We will supplement bait card monitoring with nest surveys to assess whether eradication has been achieved. Argentine ants nest exclusively under rocks at HALE, and each month we will turn over every rock within a different 10 m by 10 m quadrat in the monitoring core, scoring each nest for numbers of workers, eggs, larvae, pupae, queens and males. The central part of the monitoring core will be divided into 24 such quadrats, and one will be randomly selected each month for each plot. Only when nest surveys fail to uncover any live nests in the plots will eradication be judged to have occurred.

After a pre-treatment monitoring event, Drax Liquidator Ant Bait will be supplied in the two treatment plots, and will be deployed within commercial Drax liquid bait stations (unless these prove to be insufficiently rigorous for the environmental conditions; in this case we will use pvc bait stations developed for prior experiments). The liquid bait in these stations will be refilled or replaced monthly, as needed, over the course of one year, unless eradication is achieved sooner. We will monitor ant levels and nest survival throughout this period.

We envision this experiment as the next step forward in our research into boric acid liquid bait stations. If eradication is achieved using a 20 m bait station spacing interval, we will apply for another EUP and begin a subsequent experiment investigating 30 m and 40 m spacing intervals. At the same time, we will begin applying for a federal EUP allowing larger testing areas in order to replicate our results, design additional experiments to investigate non-target impacts, and begin the process of obtaining a Special Local Need extension to the product label for our intended use pattern. We will continue to maintain contact with other ant control specialists, and will investigate other ant baits as they become available; we may also test higher concentrations of boric acid if this appears promising.

Schedule of Activities

Oct 15, 2006 – start date
Oct-Dec 2006 – apply for state EUP, site visit by HDOA pesticides branch, acquire Drax Liquidator Ant Bait and bait stations, establish plots and bait station locations, and conduct pretreatment monitoring
Jan 2007 – place Drax bait stations and fill with Drax Liquidator Ant Bait
Jan 2007-Jan 2008 – refill bait stations as needed and conduct monthly monitoring in treatment and control plots
Mar 2008 – complete report of experiment results
Apr 2008-Oct 2008 – submit results for publication, investigate next phase of boric acid ant bait research as stated in Methods, test other products if they become available
Oct 15, 2008 – completion date

Deliverable Products

Deliverable products will be in the form of a report of experimental results, including data analysis and interpretation. If deemed worthwhile, a manuscript will be prepared for submission for publication as a PCSU or similar technical report, or in a journal such as the Proceedings of the Hawaiian Entomological Society. Results may also be reported at meetings such as the Hawaii Conservation Conference and Pacific Entomology Conference.

Personnel

The experiment will be carried out by Paul Krushelnycky, who expects to complete his doctoral dissertation at the University of California, Berkeley, by the fall of 2006. Paul Krushelnycky

worked with Argentine ant control at HALE from 1994 to 1998, during summer seasons from 2001 to 2004, and during the fall and winter of 2004/2005. He continues to research the ecology of invasive ants for his graduate work, and has maintained active contact with other ant control specialists and ant ecologists over the past decade.

Budget

HISC portion of salary and benefits (at 30%) for Paul Krushelnycky	$29,250/yr
HISC portion of equipment & supplies, including bait and bait stations	$5,330/yr
HISC portion of travel expenses	$1250/yr
Indirect costs (through PCSU or HCSU)	$4170/yr
Total	$40,000/yr
2 year Total	**$80,000**

HISC funds will be matched at least 1:1 by National Park Service funds from HALE, and will cover the balance of salary and benefits for Paul Krushelnycky, additional costs of bait and other supplies and equipment, and any additional travel expenses. In addition, NPS will provide office space and computer network resources, and USGS will provide vehicle support.

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Efficacy and palatability of commercially available rodenticides

Amount Requested $ 69,700

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PROBLEM STATEMENT

Invasive rodents in Hawaii decimate native flora and fauna, reduce agricultural production, and spread human diseases. Rodenticides are the most effective tool for broad-scale rodent control, but although many rodenticides are commercially available for use in and around buildings, only a few are approved for use in Hawaii in non-commensal areas. Furthermore, the efficacy of these products is unknown for wild rodents commonly found in Hawaii (Jacobs 1994). We propose a comprehensive laboratory study to test the efficacy and palatability of commercially available rodenticides to identify the most effective rodenticides for use on Hawaiian populations of black rats (*Rattus rattus*), Polynesian rats (*R. exulans*), and mice (*Mus* musculus). The results would then be used to support the registration of additional rodenticides in Hawaii for use in conservation areas, agricultural crops, and to protect human health.

Only two rodenticide compounds are currently approved for non-commensal use in Hawaii, diphacinone and zinc phosphide. Three products containing diphacinone were registered for conservation areas and two were registered for macadamia nut orchards; however the manufacturer of two of the products has recently cancelled these uses, leaving only a single rodenticide product available for controlling rodent damage to macadamia nuts and a second for native plants and animals in Hawaii. An additional registration for zinc phosphide is maintained by the Hawaii State Department of Health's Vector Control Branch. While these existing rodenticide registrations have proved to be invaluable, a broader array of approved rodenticides is desperately needed for many reasons: the diversification of Hawaiian agriculture has outpaced the development of new labels for rodenticides; other compounds, such as brodifacoum and chlorophacinone, may be more toxic to particular rodent species, such as mice; different bait formulations may be more attractive to rodents in Hawaii; repeated use of a single rodenticide may lead to the development of resistance to that compound in local populations; and the remaining manufacturers could also decide to cancel their registrations in Hawaii, leaving Hawaii without any rodenticides to protect native species, agricultural crops and human health.

The first step in seeking additional rodenticide registrations from the U.S. EPA and the Hawaii State Department of Agriculture is to conduct a series of laboratory trials to establish the efficacy of each compound for each rodent species. These laboratory trials will be conducted using systematic methods similar to those previously used to establish efficacy for diphacinone in Hawaii (Tobin 1994, Swift 1998). Pesticide registrations must be backed up with data sets rigorously conducted under Good Laboratory Practices (GLP). The Hawaii Field Station of the USDA National Wildlife Research Center is the only laboratory in Hawaii qualified to conduct GLP studies on rodents.

Resource Protection

Currently, only Ramik Mini-Bars (diphacinone) is registered to protect native species from rodent predation (SLN # HI-980005). A second diphacinone formulation was withdrawn from the market in Hawaii in September 2005. Thus, if this product losses its effectiveness, it is no longer palatable to rodents, or rodents develop resistance to the active ingredient, resource managers would not have an alternative available. Furthermore, the company currently producing this product could stop making it, decrease production, or fail to renew their label. For all of these reasons, additional products should be tested and evaluated.

Island Conservation

During the last 15 years, efforts to eradicate rodents from islands have increased and several successful international eradications have been completed using commercially available rodenticides (Veitch and Clout 2002). Few projects have been completed in the United States, but several projects are now in the initial planning stages, such as on Lehua Island (Hawaii). Only a single successful eradication effort has been completed in Hawaii on Mokolii Islet using diphacinone (D. Smith, Hawaii DLNR, pers. comm.). No rodenticides are currently registered for island eradication but the National Wildlife Research Center (USDA) and the US Fish and Wildlife Service have submitted a registration packet to the U.S. EPA for the registration of a 0.005% diphacinone bait pellet to be used for to eliminate rodents from offshore islands (Witmer and Eisemann 2005). A registration is also being sought for the use of 0.0025% brodifacoum pellets for the same purpose (J. Eisemann, USDA NWRC, pers. comm.).

Agriculture

In Hawaii, few rodenticides are registered for traditional crops (e.g., macadamia nuts) and many of the emerging agricultural products (e.g., tropical fruits) have no rodenticides registered. Currently, only diphacinone (SLN HI-980006) and zinc phosphide (EPA Reg. No. 12455-17) based products are registered for use in agriculture and these product are restricted to macadamia nut orchards and sugar cane. Several Hawaiian agriculture producer groups have requested registration of rodenticides for use in their crops.

Human Health and Safety

In Hawaii, rodents are known vectors of leptospirosis and murine typhus and control of rodent outbreaks with rodenticides is one way to reduce the risk of disease outbreaks. Currently, only zinc phosphide is registered to control rodent outbreaks for human health in Hawaii (SLN # HI-010001). There is considerable human health risk having only a single product registered because the product may not be effective or available when needed.

OBJECTIVE/HYPOTHESES

Due to genetic and behavioural variation between species and populations, it is necessary to screen each rodenticide and its bait matrix against each species targeted. Although all of the rodenticide bait products proposed for testing in this study are already registered for commensal use in the U.S., only diphacinone has been tested in the laboratory on wild-caught populations of rodents. It will be used in this study as a baseline compound for comparison of the results of the other compounds.

The EPA defines a rodenticide bait as being considered efficacious if 80% of the individuals in a test group consume a lethal dose. Because rodents in the wild have a choice of food items, an efficacious bait must be attractive/palatable as well as toxic. A standardized protocol will be used to determine the efficacy of each toxicant and the palatability of its bait matrix through a series of tests. Some of the compounds that will be tested are usually lethal in a single feeding, while others generally require multiple feedings to build up a lethal dose, thereby posing less risk of accidental poisoning to non-target species. These compounds will be more effective with a longer exposure period. Both single feeding and multiple feeding rodenticides would be of value for use in Hawaii.

Initially, rodenticide baits will be offered for a minimal exposure period and with alternative foods available. This will screen for the most acutely toxic and most attractive products. Additional tests will extend the exposure period and, if needed, eliminate the alternative food to evaluate potential problems with palatability and/or resistance.

We hypothesize that some commercial rodenticide baits will be found to be efficacious (≥80% mortality) to Hawaiian wild-caught rodents.

METHODS

This study is designed to be conducted in 3 tiers. Tier 2 is only conducted if rodent mortality is less than 80% in Tier 1. Tier 3 is only conducted depending on the outcome of Tier 2.

Tier 1 Study: A two-choice rodenticide efficacy trial with a 3-day exposure period.

Tier 2 Study: A two-choice rodenticide efficacy trial with a 7-day exposure period.

Tier 3 Study: A no-choice rodenticide efficacy trial with a 7-day exposure period. This study is only conducted if the Tier 2 Study is conducted and does not achieve a mortality rate of 80% or greater. The Tier 3 Study is to determine if there is a palatability problem with the bait (and it won't be consumed if an alternative food is available as in the Tier 1 and 2 Studies) or if genetic resistance to the compound occurs in the Hawaii wild rodent population.

Two-choice feeding trials (Tier 1 and 2). Free-ranging rodents, live-trapped near Hilo will be maintained in individual rack cages. The rodents will be provided with commercial laboratory rodent chow and water ad libitum. The rodents will be allowed at least three days to acclimate to the cages before the trial begins. The rodents will be weighed and sexed within a week of the start of the trials.

On day 1 of the 3-day or 7-day, two-choice feeding trial, 10 caged rodents will be randomly assigned to each treatment (0.005% diphacinone; 0.005% brodifacoum; 0.005% bromadiolone; 0.005% chlorophacinone; 0.002-5% difethialone; 0.075% cholecalciferol; 0.01 % bromethalin; 2% zinc phosphide; and 0.025% warfarin/coumarin) group; another 10 caged rodents will be assigned as the control group. All rodents will be at least 2 months of age (i.e., sexually mature and healthy). Each group will have 5 females and 5 males. The control group will continue to receive rodent chow and water. The treatment group will have the rodent chow supplemented with an assigned rodenticide bait and will continue to receive water. About 30 g of the rodenticide pellets will be added initially. Rodenticide bait and rodent chow will be replenished as needed so that rodents always have both types of food available. The position of food and bait will be randomly assigned in food cups to minimize position bias. Food consumption will be monitored by weighing food when the trial begins, as food is replenished, and any food accumulating below the wire cage on the tray. All rodenticide bait will be removed at the end of the third (Tier 1) or seventh day (Tier 2).

All rodents will be examined twice daily by the study director or his designee and the condition of the rodents and any mortalities will be recorded. Dead rodents will be placed in a labeled zip-lock bag and refrigerated for later necropsy. When necropsied, they will be weighed, sexed, and examined for signs of rodenticide poisoning as described by Stone et al. (1999). Rodents will be observed for another 10 days after the rodenticide bait is removed before all remaining rodents are euthanized and processed as described above. During the 10-day period, all rodents will be maintained on rodent chow and water. Any mortalities that occur in that 10 day period will be recorded and carcasses processed as described above.

If less than 80% of the rodents of a rodenticide treatment are dead or moribund at the end of the 10-day observation period, the Tier 2 study will be conducted with another group of 10 rodents. This trial will be conducted as described above, with the exception that the rodenticide bait will be provided for 7 days before being withdrawn and the 10-day observation period commencing.

No-choice feeding trails. Tier 3 will only be conducted if less than 80% of the rodents in a treatment group are dead or moribund by the end of the trial for the 7-day rodenticide exposure period (Tier 2). This trial will determine whether the rodenticide will be consumed and cause mortality when no alternative food is available and whether the rodents are not genetically resistant to the rodenticide (important because genetic resistance to some anticoagulant rodenticides has been found in some populations). Rodents not used in previous trials will be used and the trial will be conducted essentially the same as the above two-choice trial, except that no rodent chow will be presented until after day 7 of the trial. As before, there will also be a 10- rodent control group that is fed rodent chow throughout the trial.

Rodents will be randomly assigned to the treatment and control groups. The percent mortality of treatment groups and the control group will be compared with a Chi-square contingency test. The food consumption by groups will be compared with a multiple analysis of variance test. The pre- and post-trial weights of groups will be compared with a multiple analysis of variance test. Necropsy results will be tabulated and described by treatment.

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hampered by the unknown specific origin of *Q. erythrinae*. Therefore, an exploratory search for natural enemies of EGW must rely on a "best guess" scenario, although J. LaSalle (CSRIO, per. com.) believes EGW is of African origin. Hawaii Department of Agriculture is sending Dr. Mohsen Ramadan, Exploratory Entomologist, to Tanzania. The successful search, identification, importation, mass-rearing, host specificity studies and approval for release of an effective natural enemy in Hawaii is years away. In the meantime, other EGW management strategies must be pursued.

EGW management strategies must rely on pragmatic cultural and chemical control practices to "buy time" until a biological control solution can be developed and implemented. A preliminary systemic insecticidal trial conducted in collaboration with Takano Nakamura Landscaping Nurseries, Pearl City, Hawaii, Verdicon Hawaii (Agro-chemical distributor) and Hawaii Department of Agriculture, indicates that imidacloprid injection is effective against EGW (Fig 1). Abamectin injection, imidacloprid and dinotefuran drenches were not effective. However, reports from various state agencies, and private entities have reported that imidacloprid and dinotefuran drenches were effective against the EGW. Mr. Guy Hera, Verdicon reported that a combination of imidacloprid and abamection injections on the same tree was effective in an unreplicated trial; however, translocation of chemicals in a tree usually occurs vertically and the degree of chemical mixing is unknown. In addition, the longevity of an effective systemic insecticide against EGW in erythrina trees is not known. Translocation of systemic insecticides to flower petioles and seed pods of erythrina is also unknown. Ideally, systemic insecticide should protect seed pods from EGW and wiliwili seed beetles to ensure long term survival of wiliwili via viable seed production.

In addition to chemical controls, Maui County implemented the cultural control strategy of defoliation to help minimize the spread of the gall wasp with varying results. It is unknown how these cultural practices affected infestations given the rapid re-flushing of plants with leaves following defoliation. It is possible that defoliation may create additional stress for the plant thereby affecting the long-term health of trees.

The objective of this proposal is to develop effective management strategies for immediate implementation to preserve *Erythrina* spp. in Hawaii and minimize damage by EGW until a long term solution biological control can be successfully implemented in Hawaii. These management strategies will focus on developing EGW control options for homeowners, nursery and landscape industries, and Hawaii's native forests.

Approach

Management of *Erythrina* spp. against EGW will focus on cultural and chemical controls.

Cultural Control

Cultural control plans will focus on defoliation of leaves followed be chemical control using foliar contact insecticides and drenches/injections with systemic insecticides. Two different defoliation systems will be tested to measure efficacy in control of EGW infestations, 1) manual defoliation using pruning and 2) chemical defoliants. Registered chemical defoliants for use on ornamental plants will be sequestered by contacting major agro-chemical manufacturers. Tests

will be conducted on seedlings and/or saplings where defoliation is feasible. Following defoliation, insecticides will be applied at different time intervals to determine optimal time for chemical application for effective control of EGW. Foliar sprays of carbaryl or acephate will be tested. Systemic drenches will include imidacloprid or dinotefuran. Tissue samples will be taken and evaluated for effects of defoliant on tissues, if any, concentration of pesticides, and number of EGW produced from each sample.

Chemical Control

Chemical treatment studies will focus on foliar, drench and injection of chemical insecticides into trees. Foliar sprays and drenches will be evaluated for efficacy against EGW in seedlings and saplings where foliar sprays are feasible and safe for use. Acephate, carbaryl, abamectin milbemectin, imidacloprid and dinotefuran will be evaluated as foliar treatments and imidacloprid, acephate and dinotefuran will be also be tested when applied as a drench. All insecticides will be tested at the recommended label rates in consultation with the manufacturer. Tests will be conducted on potted erythrina plants in laboratory or green house settings. Growth conditions, including watering and light, will be adjusted to provide optimal growing conditions. Some plants will be tested with minimal watering to duplicate conditions seen in natural environments which may affect translocation of the chemicals. In addition, plants will be placed within screened cages with EGW for choice/no-choice tests. Tissue samples of all plants will be taken to measure concentration of chemicals and for number of wasps produced from each sample.

A second set of studies will be performed to evaluate management strategies on endemic populations of *E. sandwicensis* and for *Erythrina* spp. used for landscape and windbreaks. Tests will be conducted on *E. sandwicensis* at Koko Crater Botanical Garden and Pu wa'wa, Hawaii. Windbreak erythrina will be tested at CTAHR's Magoon Facilities in Manoa Valley and/or Waimanalo Research Station. Drenches and soil injections using the systemic insecticides imidacloprid and dinotefuran will be conducted at recommended label rates in consultation with the manufacturer. Preliminary data obtained by A. H. Hara and D. M. Tsuda, and anecdotal information from reputable observers indicates that drench efficacy is determined by two major factors, 1) proper placement and 2) soil moisture, and these two factors will be considered for optimal drench effectiveness. Trees will also be injected with abamectin and imidacloprid with and without irrigation using the Mauget, Wedgle, and Sidewinder injection systems. Leaf samples will be taken to determine concentration of chemicals within the leaf tissue and to count the number of wasps produced. The following will be evaluated:

- Optimal application time in relation to tree biology (i.e. before trees flush with new leaves, as new leaves begin to flush, or after flushes occur)
- Optimal application for drenches and soil injections (pre-and post- moistening soil, application at base, grid or drip canopy line)
- Duration of protection offered by drenches and injections
- Optimal number of applications per year

Management plans for large wiliwili trees (larger than 24 inch dbh) must be sustainable and cost effective as well as be safe for applicators. Injection systems will be evaluated for management of large trees and trees in forested areas. Injection systems to be studied will include Mauget,

Wedgle and Sidewinder delivering abamection or imidacloprid. Trees will be injected with and without irrigation. Dosages of systemic insecticides will be at recommended label rates in consultation with the manufacturer. Tests will be on *E. sandwicensis* at Koko Crater Botanical Garden and Pu wa'wa, Kona. Leaf samples will be taken to determine concentration of chemicals within the leaf tissue and to count the number of wasps produced. Tests will be conducted to determine the following:

- Long term effect of drilling/boring holes into trees
- Optimal application time in relation to tree biology
- Ability of plants to translocate chemicals in natural conditions with little or no rainfall
- Long term effects of chemicals remaining untranslocated in plants
- Duration of protection offered by injections

Schedule of Activities (Research Timeline)

Time (months)	Action
1-3	Plant seedlings, site selection, experimental design, procure chemicals and injectors
3-6	Install cultural and chemical experiments
6-12	Evaluate experiments, analyze data, if necessary retreat, work on progress and final report.

Description of Deliverable Products

Effective cultural and chemical control recommendations for the EGW attacking landscape erythrina and native wiliwili will be the major outcome of this research project. Effective management options for EGW will be presented at invasive species conferences, placed on web pages, and published as an outreach bulletin. Press releases on control of EGW will be made to newspapers and television broadcasters. CTAHR's Office of Communication will assist in developing, web pages, bulletins, video and press releases. Appearances on public access television and the KTA Show Hosted by Derek Kurisu will also be explored. A detailed report of results of this research project will be published in scientific, and in forestry and landscape trade journals and newsletters.

Personnel and Partners

Dr. Arnold Hara (0.15 FTE) will serve as the overall research leader and coordinator for this project. Dr. Neil Reimer, HDOA, Plant Pest Control Manager, will oversee the entire HDOA participation in this major undertaking contributing expertise in the behavior, ecology and biology of EGW. Mr. Hauff, Forest Health Coordinator, DLNR and Ms. Anne Marie LaRosa, Forest Health Coordinator, USDA Forest Service will share their expertise on the endemic wiliwili and assist in site selection, treatment and evaluation in the forest setting. Mr. Dick Tsuda, Research Support, UH-CTAHR will assist in developing experimental protocols and data analyses for cultural and chemical control strategies. Mr. Patrick Conant, HDOA Entomologist in Hilo will assist site selection and experimental design in agricultural settings. Agro-chemical representatives from HGP, Inc. and Verdicon, Inc. will provide recommendations of products for testing and for procuring appropriate injection systems. A research support personnel (1.0 FTE) and student helpers will be hired to facilitate all aspects of this research project.

Budget

The requested $98,325 will be primarily used for support personnel, operational expenses, supplies and equipment as detailed below.

		Requested
Personnel –	Research support, student help	$50,000
Supplies –	Sampling supplies, chemicals, chemical analyses*	$40,000
Travel-	Interisland airfare, U-Drive, per diem,	$ 5,000
Overhead	Grant overhead, 3.5% modified direct cost	$ 3,325
TOTAL		$98,325

* Chemical analyses is expected to account for the bulk of the supply budget. Cost per sample is estimated at $150. The sampling will be minimized but is necessary to determine proper dose rates and application times of chemicals. Sampling at 10 samples per chemical per species for a six month period is expected to cost $18,000.

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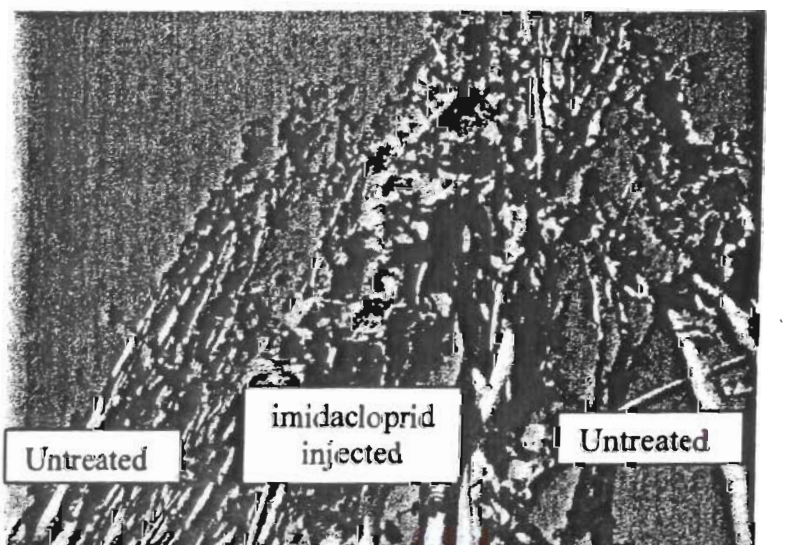

Fig. 1. Imidacloprid injected and untreated 'Tropic Coral' tall erythring 7 weeks after treatment.

Detection, Control and Phenology of the Nettle Caterpillar, *Darna pallivitta* (Moore): Applications of a Pheromone Lure

Hawaii Invasive Species Council

Research and Technology Grant Program Proposal

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Amount Requested: $20,000

Problem Statement:

THE PROBLEM: *Recent progress toward developing a pheromone lure for the nettle moth,* Darna pallivitta *(Moore), has opened new rapid detection and control options for this irritant-causing/economically-important invasive pest. However, synthesis of the lure components is not trivial and multi-gram quantities of the components are needed to conduct further field research on moth phenology and detection, and evaluate the effectiveness of pheromone mating disruption and mass trapping control for* D. pallivitta.

Infestations of the nettle caterpillar were first discovered at a nursery in Panaewa, on the eastern side of Hawaii in September 2001 (Conant et al. 2002). *D. pallivitta* is known to occur in Southeast Asia where it feeds on palms (coconut and areca) and grasses (Cock et al. 1987) and was probably introduced to Hawaii with a shipment of Rhapis palm (*Rhapis excelsa*) seedlings from Taiwan (L. Nakahara, personal communication). Initial attempts to contain the *D. pallivitta* outbreak in eastern Hawaii were not successful and caterpillars are now found in an area that includes Kurtistown, parts of Paradise Park, Hilo and the Hamakua coast. Contaminated nursery stock is a continuing source of possible outbreaks both on the Big Island and on other islands.

D. pallivitta caterpillars have a wide host range, feeding voraciously on many agricultural crops, including coffee and macadamia, as well as landscape plants thereby threatening a nursery industry valued at $97.7 million (National Agricultural Statistics Service 2004). Particularly susceptible to damage are palms, the single most valuable floriculture/nursery crop in Hawaii, and dracaenas, which were worth a combined $12.9 million in 2003 (National Agricultural Statistics Service 2004). Hawaii county accounts for over half of the flower/nursery production of the state (National Agricultural Statistics Service 2004) and faces a threat both from direct caterpillar defoliation and by lost markets, illustrated by the rejection of a recent palm shipment by California and the fumigation of another on Maui. Economic losses due to nettle caterpillar are relatively small to date, but can be expected to rise dramatically if the moth is allowed to spread throughout the Big Island and to other islands.

Additionally, the caterpillar constitutes a human health hazard due to the painful sting which results from contact with its spines. The caterpillars are small, reaching a maximum length of 2 cm, and have mottled markings, which can make them hard to see. The four longitudinal rows of spines covering the dorsal and lateral surfaces contain a toxin which is released into the skin on contact causing burning and itching. The frequency of human contact is heightened by the large host range and often high density of *D. pallivitta* caterpillars. Increased spread of the moth is likely to cause irritation for agricultural workers, homeowners, and tourists who may be stung when touching or passing infested plants.

ADDRESSING THE PROBLEM: With funding from HISC and in collaboration with researchers from UH-CTAHR and the Hawaii Dept. of Agriculture, the primary authors (USDA, Agricultural Research Service), have been able to identify a sex pheromone based lure that is effective in capturing male nettle moths (Fig. 1). Coupled gas chromatography-electroantennogram detection (GC-EAD) analysis of abdominal tip extracts revealed two male electroantennographically active compounds produced by female *D. pallivitta*. Mass spectral analysis and subsequent confirmatory synthesis identified both the major pheromone component (MPC) and minor component as (*E*)-7,9-decadienoates (Fig 2), which are structurally similar to sex pheromone components previous reported from related *Darna* sp. Additionally, a third (*E*)-7,9-decadienoate was identified from female abdominal extracts and a strong EAD response was elicited by the synthetic compound. Field trials showed significant attraction to all lures containing MPC (Fig. 1) while the minor components did not increase trap captures at the levels

and ratios tested (data not shown). Synthetic pheromone lures were also shown to outperform virgin moths at 2.5 mg/trap, demonstrating the efficiency of the lure (Fig 1).

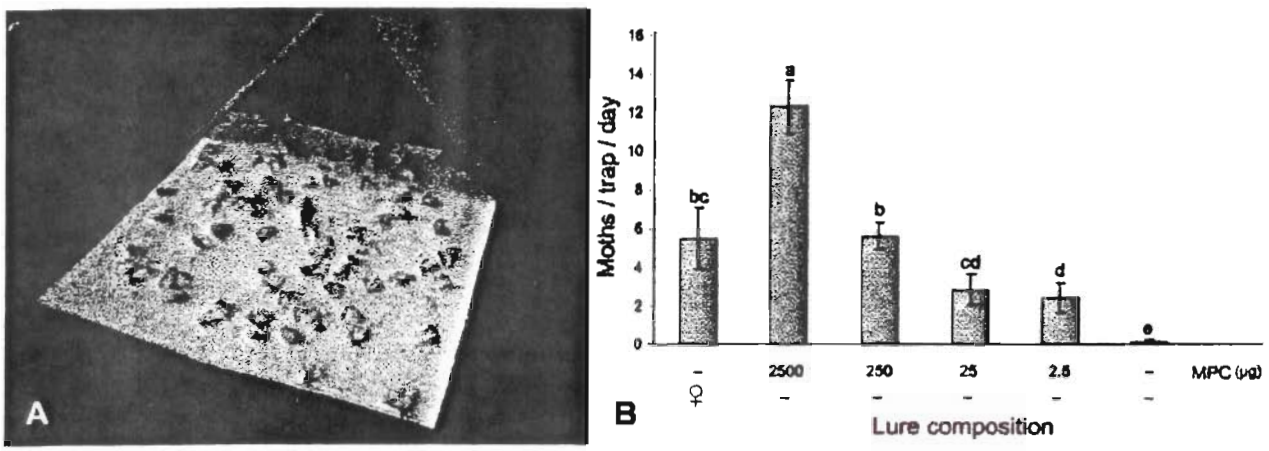

Figure 1. (A) Sample one night trap capture from Paradise Park, HI. (B) *D. pallivitta* trap captures using the major pheromone component (MPC) at varying concentrations (female moths included as a positive control).

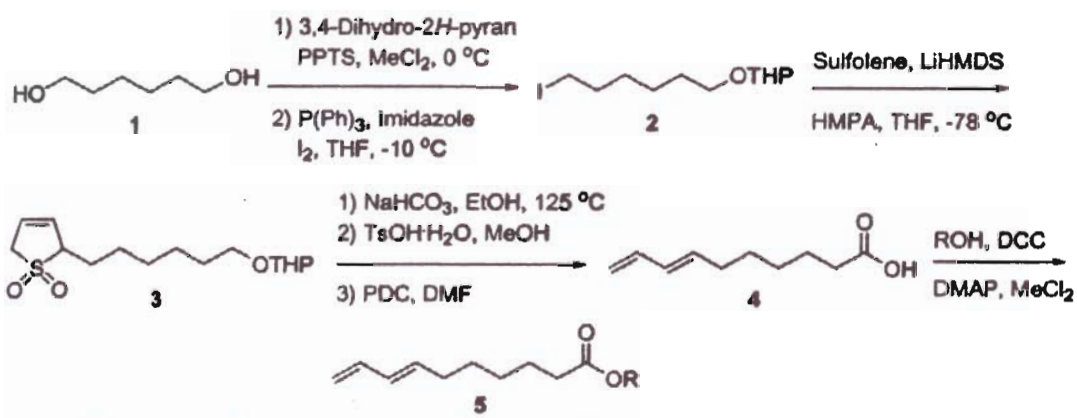

Figure 2. Synthetic scheme for *D. pallivitta* pheromone components.

While an effective pheromone lure for *D. pallivitta* had now been identified, only a small amount of the compound was synthesized (~200 mg). For more extensive research and to make the lure available to action agencies, a scaled-up synthesis from a commercial source is needed. Small chemical synthesis companies are available for this type of preparation and amount of material needed is not excessively large due to the potency of the lure (5 g of MPC = 20,000 250 µg traps). Commercial sources for the pheromone lure would also insure that action agencies could acquire lures for ongoing monitoring and control.

Multi-gram quantities of the pheromone would allow further research into the phenology of the moth and monitor incursion on other islands. Little is known about the response of the moth to the pheromone lure (calling microlocation and periodicity) and phenology information could lead to more effective deployment of detection/control traps. Grid trapping both in areas that have been infested for several years and areas that are at the edge of the moth's range could give information on population fluctuations and radiation into new areas with relation to

elevation and microclimate (compare range expansion in Paradise Park (dry, warm) to areas between Keaau and Volcano (wet, cool).

The pheromone lure may also have control applications such as mating disruption or mass trapping. Disrupting the communication and therefore the mating of adult *D. pallivitta* moths may provide an alternative to insecticide treatment. The replacement of organophosphates or other pesticides with pheromone disruption is a well documented strategy illustrated by the use of this technique to control codling moth, *Cydia pomonella* (L.), an important economic pest of U.S. mainland tree crops (Calkins 1998, Gut & Brunner 1998). Preliminary pheromone mating disruption experiments have shown a 50 % decrease in males responding to calling females with 250 µg disruption treatments. Mass trapping may be another population suppression technique that would involve the intensive use of pheromone-baited traps to capture most of the males in the area (male annihilation).

Methodology:

OVERVIEW: There will be three parts to the research with parts 2 & 3 contingent on the first. 1) Procure multi-gram quantities of the nettle moth, *D. pallivitta*, pheromone to promote further research and detection efforts. 2) Deploy pheromone traps in infested areas and throughout Hawaii to study moth phenology and detect expansion of the current geographic range. 3) Conduct field testing to evaluate the effectiveness of pheromone mating disruption for *D. pallivitta*.

COMPOUND SYNTHESIS: Preparation of (*E*)-7,9-decadienoates (~200 mg) was previously accomplished by modifications to the synthetic methods of Sasaerila et al. (2000) and Yamada et al. (1986) (Fig. 2). Briefly, *O*-tetrahydropyano-6-iodo-1-hexanol (**2**) was prepared by the monoprotection of 1,6-hexanediol (**1**) (Cossé et al. 2001) with subsequent iodination. Alkylation of 3-sulfolene resulted in the sulfolene adduction **3** which was subsequently heated to induce extrusion of SO_2. The resulting diene was easily deprotected with *p*-toluenesulfonic acid monohydrate (TsOH·H2O) to give the free alcohol which was oxidized with pyridinium dichromate (PDC) to the acid (**4**). N,N′-Dicyclohexylcarbodiimide (DCC) coupling of **4** with the appropriate alcohols, n-butanol, ethanol and methanol respectively, yielded the corresponding esters **5** (major and minor pheromone components). A scaled-up synthesis to produce multi-gram quantities of the pheromone lure will be contracted to a commercial source.

INSECTS: *D. pallivitta* pupae will be provided from the laboratory colony maintained at the Hawaii Department of Agricultural, Plant Pest Control Branch, Hilo, HI in collaboration with Dr. Hara at the University of Hawaii, Beaumont Agricultural Research Center, Hilo, HI. Female moths will then be caged in field traps as part of disruption experiments.

FIELD TRAPPING: Moth populations have been observed to peak during the summer months so field testing would be undertaken from May-Sept 2006. Grid trapping with pheromone lures will be undertaken in infested areas in eastern Hawaii to access aspects of population fluctuations and radiation into new areas with relation to elevation and microclimate. Field trials to be conducted at the USDA-ARS-PBARC site outside Hilo will aim to ascertain diel periodicity of calling behavior and microlocation of communication (e.g. vegetation height and type) by observations and varying placements of pheromones traps. Big Island studies will lead to a better understanding of moth phenology and allow better detection and control. Additionally, pheromone traps will be placed at inter-island points of interest where the nettle moth may make incursions. Points of interest would include airports, shipping ports and nurseries. Information gained from inter-island trapping will lead to a better understanding of

how many incursion events are occurring and may help to prevent establishment in areas currently unaffected by *D. pallivitta.*

PHEROMONE MATING DISRUPTION: This control technique will be evaluated with two types of experiments, point source field trials and area control. With point source trials varying levels of pheromone will be used to disrupt male moths from finding calling females. Preliminary tests with four 250 μg pheromone dispensers, placed 2 m from a caged female, showed a ~50 % decrease in responding males caught compared to a non-disrupted female. Control on a larger scale would be tested by disrupting mating in a field or nursery with a similar area used as a control. Pre- and post-treatment trapping would be used to evaluate control efficiency.

MASS TRAPPING: This suppression technique would be tested by high density trapping in a field or nursery with a similar area used as a control. Pre- and post-treatment trapping would be used to evaluate control efficiency.

Schedule of Activities:

1 Jan-May 2006: (start date) Have multi-gram quantities of pheromone lure prepared by commercial source.

May-Sept 2006: Field trapping, mating disruption (high populations) and prepare progress report.

Oct-Nov 2006: Field trapping, mating disruption (low populations)

May-Sept 2007: (end date) Conduct additional field work as needed. Prepare manuscript(s) and final report. Discuss findings with state agencies. Make presentations at scientific meetings. Plan further research with cooperators to implement findings in monitoring and control efforts.

Description of Deliverable Products:

The deliverable product from this project will be multi-gram quantities of the pheromone for *D. pallivitta* to facilitate further research on this moth by the current investigators and other researchers and agencies. Additionally, detection, phenology and pheromone disruption control studies will be undertaken with results disseminated to action agencies and the scientific community through journal articles, conference presentations and personal contacts to insure implementation of this technology.

Personnel and Partners:

Dr. Eric Jang: Research leader/research entomologist, USDA-ARS-PBARC. Supervisory and expertise role. Background in insect semiochemicals, electrophysiology and behavior. PI for identification of the *D. pallivitta* pheromone.

Dr. Matthew Siderhurst: Research entomologist, USDA-ARS-PBARC. Supervisory and day-to-day operations. Background in insect chemical ecology, natural products structural determination and organic synthesis. Co-PI for identification of the *D. pallivitta* pheromone.

Dr. Arnold Hara: Extension/research entomologist, UH Manoa. Provision, rearing and biology of *D. pallivitta* moths. Co-PI for identification of the *D. pallivitta* pheromone and has been conducting research on the nettle caterpillar since initial outbreak. Research on *D. pallivitta* includes general biology, host range and pesticide treatments.

Patrick Conant: Regulatory/research entomologist, Hawaii Department of Agriculture. Provision and rearing of *D. pallivitta* moths. Has investigated nettle caterpillar since initial outbreak. Research on *D. pallivitta* includes general biology, rearing and control.

Budget:

USDA-ARS will be providing (in-kind) over two-thirds of the cost for this proposed project, including salaries, supplies, equipment and travel. The requested $20,000 will be used for multi-gram pheromone lure synthesis, operational expenses, and research related travel including travel to disseminate the results of the project as detailed below.

		Requested	USDA-ARS (matching)
Personnel –	scientists, technicians, student help		$50,000
Supplies –	rearing supplies, pheromone synthesis, experimental supplies	$15,000	$10,000
Travel –	travel costs to deploy and monitor trap grid	$3,000	$3,000
Overhead –	ARS-USDA overhead	$2,000	
		$20,000	$63,000

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MISC

MAUI INVASIVE SPECIES COMMITTEE

A "Coqui-free" Certification Program on the Island of Maui: Using Market Incentives to Prevent Inadvertent Vectoring

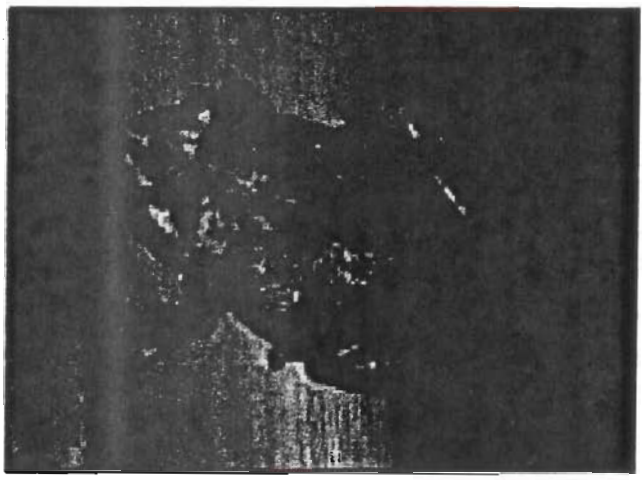

Photo: UH / CTAHR

Requested Proposal Amount: $30,000.00

Teya Penniman
Maui Invasive Species Committee
P.O. Box 983
Makawao, HI 96768
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misc@hawaii.edu

ATTACHMENT L

A "Coqui-free" Certification Program on the Island of Maui: Using Market Incentives to Prevent Inadvertent Vectoring

Problem Statement

The coqui frog (*Eleutherodactylus coqui*), native to Puerto Rico, is an invasive alien species in Hawaii. There is widespread agreement that the coqui frog poses significant environmental as well as economic threats to Hawaii. Last year alone, over $1 million was allocated for work related to coqui research and control activities in Hawaii. On Maui, the Maui Invasive Species Committee (MISC) currently has a full-time crew working daily to survey, detect, and control coqui frogs. This crew is making significant progress controlling targeted populations. However, frogs continue to arrive on Maui from Big Island plant stock and frogs continue to be moved around the island, as consumers inadvertently purchase and transport nursery stock from infested nurseries. The ultimate success – and even wisdom of – MISC's efforts depends in part on the ability to stop continued inter- and intra-island vectoring of frogs. The active and meaningful cooperation of the nursery industry is essential if Maui is to become and stay frog-free.

Unfortunately, voluntary cooperation by the nursery industry has not been shown to be an effective means for preventing the movement of invasive species (Gramling 2003; Mezitt 2003). Two reasons cited for this failure include inadequate promotion of a "do not sell" list, and lack of meaningful incentives for compliance (Caton 2005). One suggested incentive for helping to prevent the spread of invasives is a certification program for dealers in compliance with specified standards (e.g., Vickerman 1998). Other positive incentives include free advertising.

Anecdotal evidence collected by MISC suggests that local consumers want to know which nurseries have coqui frog infestations and that they will avoid purchasing from infested nurseries. Similarly, some industry participants have expressed the desire for and willingness to participate in a certification program. However, those businesses expressing interest in such a program have typically been low-volume providers. Collecting reliable information about which industry participants have frogs and getting that information to consumers has proven difficult to date. Obstacles include: gaining cooperation of nurseries; dedicating staff resources to conduct repeat surveys; and lack of a formal certification program with specific standards. Funding of this project will help overcome these challenges. Additionally, information from this project may help guide policy makers in crafting regulatory approaches by shedding light on how specific incentives may affect industry participant behavior. The information may also assist agency staff in determining whether to focus on particular segments of the industry.

This proposal requests support for development and implementation of a certification program to encourage industry participants to adopt specific practices designed to prevent the inadvertent spread of coqui frogs through the nursery trade on Maui. This program will offer incentives by allowing participants to market themselves to local consumers as certified "coqui free." We believe that MISC has adequate name recognition on Maui that certification will be viewed as meaningful. The proposed project

will include a significant public outreach / education component regarding the certification program.

The intent of this research project is threefold: 1) develop and implement a voluntary "coqui-free" certification program; 2) evaluate whether participation in a "coqui free" certification program varies by size of the business; and 3) assess the impact of the certification program and associated marketing on consumer behavior.

Hypothesis 1 (Market Incentive): Low volume businesses will be more likely to participate in a certified "coqui-free" program than high-volume businesses.

Hypothesis 2 (Consumer Behavior): Designation as a certified coqui-free business will positively influence consumer purchasing behavior.

Testing both of these hypotheses will first require development and implementation of the coqui-free certification program, including public education and outreach about the program, described in more detail below.

The Market Incentive hypothesis will be tested by introducing the program to industry participants. Pre- and post-program-introduction surveys and inspections will be conducted to determine what efforts, if any, are being undertaken to prevent the spread of coqui frogs by the industry.

The Consumer Behavior hypothesis will be tested using a pre- and post-program introduction independent market survey of the Maui population and using targeted surveys of program participant customers.

Methodology

Development & Implementation of Certification Program

This project will create specific incentives for landscape industry participants to become free of coqui frogs at all properties where they have nursery stock.

Components of the Certification Program for industry participants will include:

- Prevention: adherence to specific protocols designed to ensure that no frogs are introduced to the participant's site, e.g., using frog-free sources, conducting inspections, establishing preventive treatment methods, training of staff; and/or quarantine of new stock.

- Control: adoption of specific practices to ensure that infested plant material is not distributed from the site, e.g., disclosure to consumers, treatment of facility and surrounding area, access for inspection/surveys by MISC staff; habitat modification, and adherence to specific reporting requirements.

Marketing Components:

- Inclusion of industry members in final development of standards

- Outreach via press releases; public service announcements; and other appropriate media
- Marketing materials for businesses to indicate participation
- Targeted outreach to industry members across market segments, based on size, geography, and place in the distribution chain (grower, wholesaler, retailer)
- Inclusion of information about participants on MISC website

Surveys

Market Incentive:

- MISC will randomly select members of the industry from a comprehensive list of businesses and classify them as high or low volume according to number of sales per year.
- MISC will design and conduct pre- and post-program implementation questionnaire surveys to determine current practices to prevent establishment of coqui frogs.
- MISC will conduct pre- and post- program implementation physical surveys of the sites to assess current practices.
- The data will be analyzed to determine statistical significance.

Consumer Behavior:

- MISC will contract with an independent survey company to conduct pre- and post-program implementation surveys to evaluate consumer awareness of the coqui frog problem, awareness of the certification program, and purchasing behavior.
- The data will be analyzed to assess the impact of the program.

Note: It is anticipated that all surveys will be "Exempt" research involving human subjects, consistent with University of Hawaii protocol.

Schedule of Activities

Development and initial implementation of the certification program will take approximately six months. The start date will depend on when funding becomes available, but it is expected that project activities will commence within two (2) months of funding availability. The data collection is expected to last twelve months and all reporting will be completed within the following three months.

Deliverable Products

A progress report will be submitted at the six-month interval and a final report will be submitted within three months of the project's end, even though the certification program is expected to continue if successful. The reports will summarize all program implementation activities, including number and type of participating businesses, as well as the results of all surveys. Results will be analyzed and presented using appropriate tables and charts, maps and photographs. The emphasis of the final report will be on management implications of a coqui-free certification program.

The final report also will discuss any operational challenges, including any factors affecting willingness to participate in the certification program.

Personnel and Partners

The Principal Investigator will be Teya Penniman, MISC Manager, in close cooperation with David Duffy, Ph.D. The project will be conducted by personnel from the Maui Invasive Species Committee. MISC's Vertebrate Operations Supervisor, Adam Radford, M.A., also will assist with management of the project.

MISC and MISC partners, specifically Maui personnel from DLNR and HDOA will contribute in-kind services by assisting with implementation of the project in the field, and providing review of project results. Additionally, MISC staff will provide substantial in-kind services by assisting project participants with detection and control of coqui frogs, and by providing outreach and education-related services.

CVs for Dr. Duffy and Teya Penniman are attached.

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Budget

HISC Grant Funds Requested

• Salaries & benefits (half-time project coordinator for 1-year)	$20,624
• Independent survey	$ 5,000
• Supplies: (outreach materials, field supplies)	$ 750
• Travel/Mileage	$ 500
• Overhead, UH indirect cost (6%)	$ 1,428
• Overhead, PCSU (5%)	$ 1,698
Total Request	$30,000

Matching Contribution

Maui County Office of Economic Development	$30,000

Taxonomic Expertise for Aquatic Invasive Species Research in Honolulu Harbor as a Component of Collaborative Tropical Surveys

Principal Investigator: Lucius G. Eldredge
Bishop Museum
1525 Bernice Street
Honolulu, HI 96817

Tel: 808-848-4139
Fax: 808-847-8252
e-mail: lge@bishopmuseum.org

Amount Requested: $15,000

Taxonomic Expertise for Aquatic Invasive Species Research in Honolulu Harbor as a Component of Collaborative Tropical Surveys

Marine nonindigenous species invasions have not been investigated in tropical seas. The Smithsonian Environmental Research Center (SERC) has studied both coasts of North America. SERC has been awarded "Aquatic invasive species research: invasive species in key tropical U.S. ports—extending standardized surveys for islands and continents". The Bishop Museum is the representative institution for studies in Honolulu Harbor. Additional support is being requested to assist with the taxonomic identification of poorly known invertebrate groups.

Problem Statement:

The extent of nonindigenous species invasions in coastal marine ecosystems has become increasingly evident in recent years (Carlton, 1989; Ruiz et al., 1997). The detected increase in the rate of invasions appears to be associated primarily with transfers by shipping into ports of bays and estuaries of the continental United States (Ruiz et al., 2000). Marine systems of U.S. islands have only been well studies in Hawaii, where more than 95 invasive/cryptogenic species are documented in Pearl Harbor alone, and some 343 invasive/cryptogenic species of variably uncertain status have been recorded (Eldredge and Carlton, 2002).

Necessary data to evaluate spatial and temporal patterns of marine invasions are lacking. The data are primarily derived from literature-based synthesis of published information rather than standardized, quantitative, and contemporary field surveys. Quality and quantity of information is very uneven among sites, reflecting distinct differences in sampling methods as well as variations in search effort. There has been considerable debate about the relationship between invasions and latitude, with arguments developed both for decreasing and for increasing numbers of invasions at lower latitudes (Elton, 1958; Fryer, 1991). Some studies detected relatively few invasions at low latitudes in terrestrial, freshwater, or marine ecosystems, resulting in the hypothesis that tropical systems are less invasible than temperate zones. Higher native biodiversity in tropical ecosystems is hypothesized to create greater resistance to invasion.

On the other hand, the high diversity of tropical systems could also translate into greater numbers of invasive species (Fryer, 1991). Cosmopolitan species that are common in tropical marine systems may actually reflect early invasions associated with ship transport (especially hull fouling) (Carlton, 1996). Moreover, some tropical sites, particularly tropical islands, are highly susceptible to invasions which have a major impact on native diversity (Wilcove et al, 1998). Port systems in the Hawaiian Islands show higher numbers of invasive species

than detected in tropical Australian ports (Coles et al., 2002; Eldredge and Carlton, 2002). At least 343 species are recorded as introduced, but not necessarily established in waters of Hawaii (Eldredge and Carlton, 2002), and new records of invasive species are currently being recorded (Godwin et al., 2004). Diversity of these invasive marine species in Hawaii is high, including 287 species from major groups of invertebrates, 20 fish species, 24 algal species, and 12 species of vascular plants. Recent ship studies of ship hull fouling in Hawaii by Godwin et al. (2004) found 48 invasive and 12 cryptogenic species, including a tropical fouling community that is rich in invasive species, including several new records.

Thus, Hawaii may illustrate alternative hypotheses to a diversity gradient effect on invasions. It may be that island systems differ significantly from continental systems in susceptibility to invasions (Wilcove et al., 1998). Also tropical and temperate latitudes may differ significantly in their historical patterns of trade and development, suggesting that the vectors/transport mechanisms for introduction have not been equivalent between regions. Thus, the number of invasions in Hawaii may result from the large amount of commercial and military shipping to these islands, and barges and dry docks also appear to be important transfer modes (Coles et al., 1999; Eldredge and Carlton, 2002).

The Smithsonian Environmental Research Center's (SERC) Marine Invasions Laboratory is a national center of research excellence on nonindigenous species in coastal ecosystems. Its extensive research includes the development of national databases of marine and estuarine invasions in North American coasts. A key component of the program has been the establishment of a broad-based program of sampling invasive species in bays and port systems in North America using standardized sampling methods for fouling communities, as well as rapid assessment surveys. SERC has been awarded a grant "Aquatic invasive species research: invasive species in key tropical U.S. ports—extending standardized surveys for islands and continents" from the National Sea Grant Program. This activity will extend their studies to provide an important latitudinal extension to the tropics at four key sites in the Caribbean Basin and Pacific Ocean where both the volume and shipping and the Panama Canal may focus the potential for introduction of species into tropical locations The proposed sites are Belize City, Belize; Honolulu Harbor; Indian River Lagoon, Florida; Panama Canal, Panama City; San Juan Harbor, Puerto Rico. The Bishop Museum is the focal point for the Honolulu Harbor portion of the study. Most studies of marine invasive species in the Western Hemisphere have focused on temperate latitudes of continental shores, with very little analysis of the tropical and island systems. SERC's extended tropical and subtropical surveys using standardized field approaches, analysis, and information integration will serve to identify patterns and test hypothesis about causes and impacts of invasions in tropical vs. temperate coastal systems, as well as fragile island vs. continental systems.

Approach:

Standardized "settling plates" and wooden blocks will be deployed at all sites—the Honolulu Harbor sites are to be determined—which have been widely used to compare coastal invasions of fouling and wood-boring communities of North America. The surveys will be conducted between May and August. The summer sampling will continue for consistency with comparative data. At each site, the fouling community will be sampled by using 200 settling plates and boring organisms will be sampled with 20 wooden blocks. The sampling units will be in a stratified, random design. Within each site, sampling will be partitioned among 10 strata with 20 fouling plates and 2 wooden blocks in each stratum. This allows for compare relative abundance of invasive and native species among strata within bays, as well as to test for differences among survey sites. Teams of researchers will participate in the retrieval of the fouling plates and wooden blocks. The team will be composed of technicians, investigators, several expert taxonomists, and students. Settling plates and wooden blocks will be processed in the field. A single plate/block may be encrusted with as many as 50 species, especially bryozoans, hydroids, tunicates, sponges, barnacles, and algae and wood-boring isopods (gribble) and bivaives (shipworms). Species are scored as "presence/absence" per plate and percent cover by point-intercept methods. A voucher specimen will be collected for each morpho-species on each plate/block. Averaging 25 species/plate and 3 blocks will require 5,060 species identifications and vouchers per site. Voucher specimens will be identified by specialist taxonomists and will be deposited in the collections at the Bishop Museum. With such a diversity of organisms, we are herein requesting taxonomic support to assist with the poorly known invertebrate groups—polychaet annelids, sponges, bryozoans, etc.

Schedule of activities:

Initial background and site selection will take place during the spring of 2006; the settling plates will be put in place and retrieved during the summer of 2006. The taxonomic support will be time consuming and detailed, taking many hours of specialist investigations.

Deliverable products:

Through the specialist taxonomists, a finite identification will be made of all the species collected. This will aid in the identification of the biota and will assist managers in making determinations of species that may not occur here through a process of elimination of what is already in Honolulu Harbor. Along with the results of the other settling plate surveys, a comprehensive review of the related tropical sites will be compiled.

Personnel and Partners Budget:

The Smithsonian Environmental Center's Marine Invasions Laboratory is a national center of research excellence on nonindigenous species in coastal ecosystems. SERC's extensive research includes development of national databases on marine and estuarine invasions for North American coasts.

Investigations will work cooperatively with Hawaii's Division of Aquatic Resources alien invasive species program.

Budget:

Salaries	$9134.86
Fringe	3631.10
Overhead (17.5%)	2234.04
Total	$15,000.00

Matching funds will originate from the SERC grant which has already been approved by the U.S. Sea Grant Program.

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Hawaii Invasive Species Council Proposal

Project title:
Survey for potential biological control agents for *Miconia calvescens* in southern Mexico

Amount requested:
$14,625

Principal Investigator:
Dr. Alec McClay
McClay Ecoscience
15 Greenbriar Crescent
Sherwood Park
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Problem statement

Miconia calvescens is a tree in the family Melastomataceae, native to rainforests of tropical America, which has become a major invader of native forest ecosystems in several Pacific island groups. It has become dominant over 65% of the island of Tahiti, displacing native forest communities and threatening up to 100 native plant species (Meyer 1996). *M. calvescens* was found to have escaped from cultivation on Maui in 1990, and efforts to contain established populations have been under way since then. Invasive populations also have been targeted for control on Hawaii, Oahu and Kauai. It is considered to represent a threat to all habitats in Hawaii receiving more than 1800 mm annual precipitation (Loope 1997).

Studies on potential biological control agents for *M. calvescens* were initiated in 1993 with surveys in Costa Rica, Brazil, Uruguay, Paraguay, Argentina, and Trinidad and Tobago. Currently the US Forest Service is coordinating studies of several potential biocontrol agents in Costa Rica and Brazil.

The native range of *M. calvescens* extends from Brazil to southern Mexico (Wurdack 1980). According to Meyer (1998), Mexico was the source of *M. calvescens* plants cultivated at the Peradeniya Botanic Gardens in Sri Lanka, from where they were introduced to Tahiti and probably to other Pacific islands. Thus, Mexican populations of *M. calvescens* may be the most closely matched to the invasive biotypes in the Pacific islands, making them particularly suitable as a source of biocontrol agents. However, no surveys have been conducted of the natural enemies of *M. calvescens* in Mexico. From herbarium records at the California Academy of Sciences, *M. calvescens* sites have been identified in the municipalities of La Trinitaria, Ocosingo, and Palenque (state of Chiapas) and Santa Maria Chimalapa (state of Oaxaca).

We propose to survey the insects, mites, nematodes and pathogens associated with *M. calvescens* at sites where it has been reported to occur in the Mexican states of Chiapas and Oaxaca. The objectives are to determine:

1. Are there substantial populations of *M. calvescens* in southern Mexico that could harbor natural enemies?
2. Are *M. calvescens* populations in Mexico morphologically and genetically similar to the invasive populations present in Hawaii and other Pacific islands?
3. Are there additional natural enemy species present in these populations that would expand the pool of potential biocontrol agents beyond that currently known from Brazil, Ecuador, and Costa Rica?
4. Are local collaborators and facilities available for further studies on potential biocontrol agents?
5. Would further studies based in Mexico be warranted and what costs would be involved?

This study will extend and complement the ongoing studies on biological control of *M. calvescens* by providing information on the natural enemy complex attacking this plant in the northern end of its native range, which has not previously been surveyed for potential biological control agents. Comparison of *M. calvescens* populations in southern Mexico with the invasive populations in

Hawaii will help to indicate whether Mexico may have been the source area for these populations, as has been suggested (Meyer 1998).

References:

Loope, L.L. 1997. HNIS Report for *Miconia calvescens*: a product of the Hawaiian Ecosystems at Risk Project.

Meyer, J.-Y. 1996. Status of *Miconia calvescens* (Melastomataceae), a dominant invasive tree in the Society Islands (French Polynesia). *Pacific Science* **50**: 66-76.

Meyer, J.-Y. 1998. Epidemiology of the invasion by *Miconia calvescens* and reasons for a spectacular success. pp. 4-26 *in* Meyer, J.-Y. and C.W. Smith (Eds.) Proceedings of the First Regional Conference on Miconia Control, August 26-29, 1997, Papeete,Tahiti

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Methodology

The survey will be conducted by Dr. Alec McClay over a 14-day period in July 2006. The timing is designed to be approximately one month after the normal start of the rainy season in southern Mexico, so that *M. calvescens* should be growing actively and populations of natural enemies should be increasing. Facilities of El Colegio de la Frontera Sur (ECOSUR)[1] in Tapachula, Villahermosa, and San Cristobal de las Casas will be used as bases for the field survey.

Sites in the states of Oaxaca and Chiapas where *M. calvescens* has been recorded from herbarium data will be visited, and similar habitats in the region will be explored to locate any currently existing *M. calvescens* populations. GPS coordinates of all study sites will be recorded to facilitate future return visits, and sites will be documented by digital photography. Numbers and approximate sizes of *M. calvescens* plants will be recorded. Plants will be examined for arthropod, nematode, and pathogen natural enemies and for signs of damage such as defoliation, galls, leaf rolls, stem mines, etc. Natural enemies and their damage will be documented by digital photography and specimens of natural enemies will be collected for identification. Immature stages will be reared out where possible or left with cooperators at ECOSUR for rearing to the adult stage. Preserved material will be shipped to project cooperators Dr. Paul Hanson at the University of Costa Rica and Dr. Robert Barreto at the Federal University of Vicosa in Brazil, for comparison with species previously collected and identification by specialists. Preserved material of *M. calvescens* from all sites will be sent to USFS for genetic and morphological comparison with invasive Pacific populations of this species.

Dr. Pablo Liedo, Director General of ECOSUR, has expressed willingness of ECOSUR to cooperate with the project, and to provide facilities during the survey, assign staff members to accompany the consultant in field exploration, and to facilitate collecting permits. An entomologist

[1] ECOSUR is a public, multidisciplinary research and postgraduate training institution focussed on the sustainable development of Mexico's southern border states, with programs in Conservation of Biodiversity, Society, Culture and Health, and alternative production systems. It has five campuses, each with laboratory and library facilities, biological collections, and staff including botanists, entomologists, and mycologists. See www.ecosur.mx.

at ECOSUR in Tapachula has expressed interest in cooperating with the survey and accompanying the consultant in field exploration. During the visit discussions will be held with ECOSUR administrators and staff on the possibilities of conducting further work on natural enemies of *M. calvescens* at ECOSUR, the availability of facilities and personnel, and on the costs involved.

Schedule of activities

January – May 2006	Preparations for survey: make travel arrangements (flights and rental vehicle), purchase supplies and equipment, make detailed survey plans in consultation with ECOSUR staff.
July 2006	Travel to Mexico, 2-week field survey in cooperation with ECOSUR staff.
August 2006	Submission of progress report.
October 2006	Submission of final report.

Description of deliverable products

A report will be provided detailing all sites visited with the status of *M. calvescens* populations located and with a listing of natural enemies found at each site and the type and extent of damage they caused. Provisional identifications will be provided where possible. The report will compare the natural enemy complex found in Mexico with that known from studies in Costa Rica, Ecuador, and Brazil, and discuss the potential contribution to successful biocontrol of *M. calvescens* from biocontrol agents found in southern Mexico. Recommendations will be made on the desirability and feasibility of further collaborative studies in Mexico. Biological control workers in Hawaii (Tracy Johnson at the FS Hawaii Volcanoes National Park Quarantine Facility, and entomologists and plant pathologists at the Hawaii Dept. of Agriculture) will use this survey to prioritize development of biological control agents to focus on those with the highest potential for effective control.

Personnel and partners

Dr. Alec McClay:	Biological control consultant
Dr. Tracy Johnson:	Entomologist, US Forest Service Institute of Pacific Islands Forestry, Volcano, Hawaii.
Dr. Pablo Liedo:	Director, El Colegio de la Frontera Sur (ECOSUR), Tapachula, Mexico
Dr. Benigno Gómez:	Entomologist, ECOSUR

Budget

From HISC

Air fare Edmonton-Tapachula	1	ea.	@	$1,500	$1,500
Taxis/local transportation	4	ea.	@	$40	$160
Hotel accommodation	14	days	@	$60	$840
Meals/subsistence	14	days	@	$25	$350
Vehicle rental	14	days	@	$120	$1,680
Gasoline	200	litres	@	$0.65	$130
Expenses for local assistants	10	days	@	$85	$850
Consultant charges	14	days	@	$637.50	$8,925
Supplies and equipment				$350	$350
Telephone, fax, mail, shipments				$80	$80
Total (all amounts US $)					$14,625

Travel includes round trip airfare for the consultant from Edmonton, Canada, to Tapachula, 14 days vehicle rental, gasoline, meals and accommodation. Meals and accommodation for ECOSUR staff accompanying the consultant in the field are also included. Amounts are based on estimates in October 2005; actual amounts billed will reflect savings if it is possible to book accommodation, vehicle rental and air fares at lower rates than estimated.
Supplies includes consumables for collecting (bags, vials, nets, alcohol, notebooks, maps, etc.) as well as mail, telephone, fax, and shipment costs for collected material.
Contracting includes 14 days of consultant Dr. Alec McClay's time at Can$750 per day (currently approximately US$638 per day).

Matching and in-kind resources

Staff time provided by ECOSUR	10 days@$150	$1,500
Dr. Tracy Johnson coordinating species identifications and evaluating Mexico survey results (USFS)	5% time	$3,500
Dr. Paul Hanson and Univ. Costa Rica entomological staff working on miconia project funded by NPS, USGS	2006 budget	$20,000
Dr. Robert Barreto and Fed. Univ. Vicosa staff working on miconia pathogens with funds from NPS, USGS	2006 budget	$15,000
Total match		$40,000

Alec McClay: Curriculum vitae

Education:	Ph.D.	1978	University of Cambridge	Zoology
	B.A.	1974	University of Cambridge	Natural Sciences

Employment history:

2004 –	Proprietor, McClay Ecoscience, Sherwood Park, Alberta
1984 – 2004	Research Scientist, Alberta Environmental Centre/Alberta Research Council, Vegreville, Alberta, Canada.
1978 – 1983	Entomologist, Commonwealth Institute of Biological Control, based in Monterrey, Mexico.

Experience:

In my first position, with the Commonwealth Institute of Biological Control (now CABI Bioscience), I spent 5 years in Mexico conducting exploration and host-specificity testing for biological control agents for the weed *Parthenium hysterophorus* on behalf of the Queensland Department of Lands. This involved field work in many parts of Mexico. Since then I have also done consulting work in Mexico for the Canadian International Development Research Centre, including exploration for natural enemies of the leucaena psyllid and a review of their project on the biological control of coffee berry borer, and for the Alan Fletcher Research Station, Queensland, to collect further biological control agents for parthenium weed. I speak, read and write fluent Spanish.

At the Alberta Environmental Centre (which later became part of the Alberta Research Council) I was responsible for biological control research on introduced weeds of agricultural and environmental importance in Alberta. This includes selection of target weeds, exploration for natural enemies in the native range of the weeds, selection of potential biological control agents, biological studies and host-specificity screening of candidate agents to ensure their safety for field release, submission of petitions for approval of agents to regulatory authorities, importation, rearing and field release of agents, evaluation of their establishment, dispersal, and impact in the field, and development of systems to distribute promising agents to users in the field. I cooperated extensively with European-based researchers on the selection and screening of agents, with provincial and municipal extension staff on the field release and distribution of agents, and with academic researchers on the ecology, behaviour, and systematics of the biological control agents. This work resulted in the establishment of eleven insect and mite species as biological control agents against seven noxious weeds.

I gave invited presentations at the First and Second Latin American Short Courses on Biological Control of Weeds, in Nicaragua in 2002 and 2004, and after the second of these made went to Costa Rica to meet with Dr. Tracy Johnson of the US Forest Service and staff working on the Miconia biocontrol project, and to visit some of their field sites.

Since 2004 I have been working as an independent consultant in the fields of biological control, invasive species, and insect-plant ecology.

Proposal title:
Preliminary exploration for arthropod natural enemies of *Rubus ellipticus* in China

Requested amount: $12,000

Principal investigators:
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Problem statement

Rubus ellipticus, yellow Himalayan raspberry, arrived in Hawaii around 1961 and began to naturalize in the vicinity of Volcano (Wagner et al. 1990). A single plant can grow into a large, 4m tall impenetrable thicket, with a main stem exceeding 10 cm in diameter. Its recurved prickles and sturdy stems make it extremely unpleasant for livestock and humans alike. It has been spread by birds and humans to the outskirts of Hilo and as far as Laupahoehoe. Transport to Maui has been documented on hapu'u trunks and remains a great concern for land managers there. In addition to being a weed of pastures and disturbed areas, it demonstrates an alarming ability to invade deeply into pristine wet forests such as the Olaa Tract of Hawaii Volcanoes National Park. Plants can establish in dense shade and grow to overtop supporting hapu'u. Mechanical and herbicidal control has been performed on a limited scale, for example at Keahou Ranch and in HAVO. However, large scale eradication or even containment is not feasible, because of the extensiveness and rugged terrain of the infested area. High densities of young plants around Volcano clearly demonstrate the explosive population growth of *R. ellipticus*. This plant is widely recognized as a major threat to native Hawaiian forests (Smith 1985, Jacobi and Warshauer 1992).

During the 1960's the Hawaii Department of Agriculture targeted Florida blackberry, *Rubus argutus*, for biological control, releasing five insect species collected in North America (Julien and Griffiths 1998). Three of these species eventually became established through deliberate introductions on the islands of Hawaii, Maui, and Kauai. Two lepidopteran leaf-feeders, *Schreckensteinia festaliella* and *Croesia zimmermani*, were credited with significant control in pasture areas, but had limited impact in forests (Julien and Griffiths 1998, Nagata and Markin 1986). Low efficacy of the sawfly, *Priophorus morio,* was attributed to a virus with which it may have been introduced (Markin and Nagata 1995). Both native species of *Rubus*, *R. hawaiiensis* (akala) and the rare *R. macraei*, and the alien *R. ellipticus* have been found attacked by these introduced biological control agents (Gagne 1972, Nagata and Markin unpub. data), however impacts of the agents appear to be limited. In a recent evaluation of sympatric populations of *R. hawaiiensis*, *R. argutus* and *R. ellipticus*, the biological control agents *S. festaliella* and *C. zimmermani* were common at most sites on all three host plants, but larval densities on were approximately three times greater on *R. argutus* than either *R. hawaiiensis* or *R. ellipticus* (T. Johnson and R. McGuire, unpub. data).

Any additional natural enemies of Rubus proposed for introduction to Hawaii will have to be carefully evaluated for risk to native akala. Fortunately, Hawaiian *Rubus* spp. do not appear to be closely related to *R. ellipticus*, so prospects for finding at least some agents that are specific to the latter seem reasonable.

Rubus ellipticus var. *obcordatus* is native to the Himalayan region (southern China to northern India). In China its fruit are not valued highly, but leaves and twigs are used medicinally. Dr. Don Gardner (USGS PIERC, Honolulu) initiated collaborations with Chinese researchers several years ago in search of pathogens for *R. ellipticus*, but retired without finding a promising pathogenic agent. Following his experience, he concluded that insects, which were observed on *R. ellipticus* during the course of his collaborations, were more perhaps more promising than pathogens in southern China (pers. comm.). This possibility has not yet been pursued. We propose to conduct

preliminary exploration for *R. ellipticus* arthropods in China and determine where and how additional targeted studies should be pursued.

Work Plan

Objectives:

1) Identify prospective biocontrol agents and source areas from Chinese literature
2) Locate source populations of *Rubus ellipticus* and associated arthropods in southwestern China
3) Evaluate life histories and biocontrol potential of 1-2 selected insect species
4) Identify collaborators and plan in-depth studies at field sites in China

Jianqing Ding and colleagues have nearly completed a database of natural enemies of Rubus species (as well as dozens of other North American weeds of Asian origin) in China drawn from reviews of Chinese scientific literature. This database includes a list of over 40 insects species (Table 1), a few of which have potential for biocontrol. Two of these species were recorded from *R. ellipticus*. Guided by this initial review, which was drawn primarily from entomological and botanical catalogs, we will search the Chinese primary literature for more specific information on *R. ellipticus* and potential agents.

Literature will be used to plan surveys for *R. ellipticus* and associated arthropods in the provinces where *R. ellipticus* is native: Guangxi, Guizhou, Sichuan, Tibet, and Yunnan (Lu and Boufford 2003). Our survey trips will focus on identifying sites with populations of *R. ellipticus* that can be expected to support substantial populations of specialist arthropods. Preliminary surveys of arthropods will be conducted, with particular attention to relocating specialist feeders that have been previously collected. Representative specimens of plants and insects will be collected and preserved. One or two insect species of interest will be collected for attempted rearing in the laboratory at Wuhan, China.

An additional goal during field surveys will be to contact potential local collaborators. If intensive field studies are needed at a particular site in the future, competent local collaborators will be a critical asset.

Schedule of activities

Month	Activity
Mar-Apr 2006	In-depth review of Chinese literature
May-Oct	Survey for R. ellipticus and associated arthropods
Oct-Nov	Identify sites and local collaborators for intensive studies
Oct-Dec	Preliminary lab studies of potential agents
Jan-Feb	Write-up results and proposal for focused studies

Deliverables

Based on recent observations by Don Gardner and his collaborators, as well as records from the Chinese entomological literature, we are confident of finding populations of *Rubus ellipticus* with associated insects in southwestern China. We expect to collect and obtain preliminary biological data for one or two species of potential agents. Most importantly, this initial exploration will identify sites and collaborators for future detailed studies of selected potential biocontrol agents. Detailed ecological studies in the native range of *R. ellipticus* will

be critical to identify those agents likely to have significant impact on the weed in Hawaii and that are sufficiently specific to pose low risk to our native Rubus species.

Personnel and Partners

The project will be managed by Tracy Johnson (research entomologist, USDA Forest Service, Volcano), in collaboration with Jianqing Ding, of the Institute of Biological Control, China. Dr. Johnson has overseen foreign and Hawaii-based development and evaluation of insects for biocontrol of miconia and other forest weeds over the past five years, and has 20 years of research experience in insect-plant interactions. Dr. Jianqing has led research in exploration and evaluation of insects for weed biocontrol in China and the US for over 10 years. He will be establishing a biological control laboratory in Wuhan, China in early 2006. This location in central China will be within reasonable travel distance of study sites across southwestern China.

Budget

	HISC funds requested	Forest Service match
Salary for Dr. Jianqing and assistants	3,000	2,000
Travel in China	6,500	2,000
Supplies	663	
Project oversight		8,000
Indirect costs (Wuhan)	1,129	
Indirect costs (Forest Service)	708	
Total	$12,000	$12,000

Literature Cited

Gagne, W. 1972. Notes and exhibitions. Proc. Hawaiian Entomol. Soc. 21: 158-159.

Jacobi, J. D., and F. R. Warshauer. 1992. Distribution of six alien plant species in upland habitats on the island of Hawai`i. *In* Alien Plant Invasions in Native Ecosystems of Hawai`i: Management and Research, pp. 155-188. C. P. Stone, C. W. Smith, and J. T. Tunison (eds.). Cooperative National Parks Resources Studies Unit, Honolulu.

Lu Lingdi (Lu Ling-ti) and David E. Boufford. 2003. *Rubus* Linnaeus. in: Wu Zhengyi and Peter H. Raven, eds. *Flora of China. Vol. 9 (Pittosporaceae through Connaraceae)*. pp. 196-288. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis. 496p.

Markin, G. P., and R. F. Nagata. 1995. Failure in Hawaii of the sawfly, *Priophorus morio* (Hymenoptera: Tenthredinidae), a biological control agent for *Rubus argutus*, due to a virus. In Delfosse, E. S., and R. R. Scott (eds.). Proceedings of the VIII International Symposium on Biological Control of Weeds, 2-7 February 1992, Canterbury, New Zealand. DSIR/CSIRO, Melbourne, pp. 309-312.

Nagata, R. F., and G. P. Markin. 1986. Status of insects introduced into Hawaii for the biological control of the wild blackberry *Rubus argutus* Link. Proceedings of the Sixth Conference in Natural Sciences, Hawaii Volcanoes National Park. Cooperative National Park Resources Studies Unit, University of Hawaii at Manoa.

Smith, C. W. 1985. Impact of alien plants on Hawai`i's native biota. *In* Hawai`i's Terrestrial Ecosystems: Preservation and Management, pp. 180-250. C.P. Stone & J.M. Scott (eds.) Cooperative National Parks Resources Studies Unit, Honolulu.

Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1990. Manual of the Flowering Plants of Hawaii. Bishop Museum Special Publication 83. University of Hawaii Press, Honolulu.

Table 1. Arthropod natural enemies of Rubus in China.

Order	Family	Species	Specificity
Coleoptera	Buprestidae	*Coraebus quadriundulatus* Motschulsky	mo
	Chrysomelidae	*Aphthona howenchuni* (Chen)	oo
		Batophila impressa Wang	oo
		Chaetocnema simplicifrons (Baly)	oo
		Phaedon fulvescens Weise	oo
	Curculionidae	*Enaptorrhinus convexiusculus* Heller	po
	Eumolpidae	*Basilepta leechi* (Jacoby)	po
		Basilepta ruficolle (Jacoby)	po
		Chlamisus indicus Jacoby	oo
		Chlamisus latiusculus Chûjô	mo
		Chlamisus ruficeps (Chen)	po
		Chlamisus semirufus (Chen)	po
		Chlamisus setosus (Bowditch)	m†
	Hispidae	*Alledoya vespertina* (Boheman)	po
Hemiptera	Coreidae	*Derepteryx fuliginosa* (Uhler)	po
		Derepteryx lunata (Distant)	po
	Pentatomidae	*Amyntor obscurus* (Dallas)	po
Homoptera	Aphididae	*Acyrthosiphon rubiformosanum* (Takahashi)	po
Lepidoptera	Geometridae	*Dysstroma cinereata* (Moore)	mo
		Dysstroma citrata (Linnaeus)	po
		Mesoleuca albicillata (Linnaeus)	po
		Photoscotosia miniosata (Walker)	m†
		Plagodis dolabraria (Linnaeus)	oo
	Hesperiidae	*Abraximorpha davidii* (Mabille)	mo
	Lycaenidae	*Sinthusa chandrana* (Moore)	mo
	Noctuidae	*Acronicta rumicis* (Linnaeus)	po
		Anaplectoides prasina (Denis & Schiffermüller)	po
		Anomis mesogona (Walker)	oo
		Grammodes geometrica (Fabricius)	po
		Grammodes stolida (Fabricius)	po
		Sypnoides picta Butler	po

Order	Family	Species	Specificity
	Nymphalidae	*Argynnis paphia* (Linnaeus)	po
		Brenthis daphne (Denis & Schiffermüller)	po
		Brenthis ino (Rottemburg)	po
	Saturniidae	*Loepa damaritis* Jordan	po
	Tortricidae	*Adoxophyes orana* Fischer von Röslerstamm	po
		Ancylis comptana (Frölich)	po
		Archips xylosteana (Linnaeus)	po
		Epiblema tetragonana (Stephens)	po
		Epinotia ustulana Hübner	oo
		Olethreutes lacunana (Denis & Schiffermüller)	po
		Orthotaenia undulana (Denis & Schiffermüller)	po
		Syndemis perpulchrana (Kennel)	po

† Hosted by *Rubus ellipticus* var. *obcordatus*

m, mo = recorded on one Rubus species only
oo = recorded on multiple species within the genus Rubus
po = recorded on Rubus and other genera

Proposal title:
Evaluation of pathogens in Costa Rica for biocontrol of *Miconia calvescens*

Requested amount: $7,700

Principal investigator:
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Problem statement

Miconia calvescens is widely recognized as the most threatening invasive plant in Hawaii today (Medeiros et al. 1997, Meyer 1998). Although significant progress has been made in containing its spread using herbicides and hand-pulling, complete eradication of populations established in remote rugged terrain is not feasible. Introduced biological agents are necessary for long term control (Smith 2002). Classical biocontrol of weeds is the deliberate introduction of exotic herbivores and pathogens to reduce population density of an alien weed, and entails exploration in the weed's native range, evaluation of prospective agents for specificity and efficacy against the target weed, and release and monitoring of agents that meet regulatory approval (Huffaker and Messenger 1976). To date only one agent, the fungus *Colletotrichum gloeosporioides miconiae*, has been established in Hawaii for biocontrol of miconia. This leaf spot fungus was introduced from Brazil in 1997 and has been dispersed artificially and naturally (Killgore 2002), however sustained control by this single agent appears unlikely. Its impact has been rather low, causing some defoliation, but having no effect on survival or growth in an ongoing controlled study (Denslow unpublished data).

Miconia calvescens is native over a very large geographic range extending from southern Mexico to southern Brazil. To date miconia has been located and searched for natural enemies in Costa Rica, Guatemala, Ecuador and eastern Brazil, however intensive studies have been conducted only in Costa Rica and Brazil. A variety of insect species with potential for biocontrol are under study in Costa Rica and Brazil (Picanco et al. 2005), and recently in the Hawaii Volcanoes National Park Quarantine Facility. A variety of miconia pathogens, mainly fungi, have been identified by Robert Barreto, who has directed intensive evaluations of pathogens occurring in Brazil (Barreto et al. 2005). Barreto also has sampled miconia pathogens in Costa Rica and Ecuador, but only in relatively brief collecting trips.

Coccodiella miconiae is a fungus that has been considered an excellent potential agent for miconia biocontrol for several years (Killgore 2002, Barreto et al. 2005). It causes severe rust-like symptoms on leaves: yellowing and black pimples. It is an obligate parasite (cannot be raised on artificial media) and is very probably highly specific. Heavy hyperparasitization and difficulties achieving sporulation hampered its study (Seixas et al. 2005), but a reliable method for germinating spores in the lab has been discovered in the last year. Unfortunately, populations of this fungus studied in Brazil infect Brazilian miconia plants but do not infect plants from Hawaii. This extreme specificity is understandable given the great genetic diversity within *Miconia calvescens*. Observations by Barreto suggest that purple-leaved miconia plants matching Hawaii's are found mainly from Central America southward on the Andes (Ecuador and Bolivia), whereas green-leaved plants are found in eastern Brazil. There has been no genetic study to match our invasive biotype with populations in its native range, however Meyer (1998) traced the origin of the *Miconia calvescens* invading Pacific islands to Mexico. Thus there is reason to expect that miconia pathogens from Central America will be better adapted to Hawaiian miconia than pathogens from Brazil. *Coccodiella miconiae* is relatively common in Costa Rica on plants that appear to match the Hawaiian biotype, but pathotypes from Costa Rica have not yet been tested on Hawaiian plants.

Ditylenchus sp. is a recently discovered nematode that produces galling of shoots, leaves and inflorescences, often causing severe deformity in various species of *Miconia*. Its damage resembles that of tiny eriophyid mites, which were previously assumed to be responsible for

these galls. This nematode is now being studied in Brazil and methods for propagating it on potted plants have been developed. Brazilian strains of *Ditylenchus* may be found suitable for Hawaiian miconia, but *Ditylenchus* also has been collected from *Miconia calvescens* in Costa Rica, opening another opportunity for improved host matching.

In addition to possessing miconia that appears to closely match Hawaii's, Costa Rica is a favorable source of biocontrol agents because its native forest habitats with miconia are extensively conserved, much more so than any other country in the region. Our entomological collaborators at the Universidad de Costa Rica have identified a variety of field sites, and over the last five years developed the logistical capacity for intensive field studies and greenhouse experimentation with miconia. However, they lack expertise in plant pathology. There is a clear need to export experience accumulated in the Barreto lab to Central America. We currently have an opportunity to do so in the form of a young German researcher eager to pursue a 6-month thesis project on miconia pathogens in Costa Rica. With a modest commitment of additional resources she can be trained in techniques in Barreto's lab and use them to help deliver *Coccodiella* and/or *Ditylenchus* agents to Hawaii.

Work Plan

Objectives:

1) Collect and propagate *Coccodiella* and *Ditylenchus* from field sites in Costa Rica.
2) Test pathogenicity of collected pathotypes on plants of the Hawaiian biotype of miconia.
3) Document courses of infection and impacts on greenhouse plants.
4) Evaluate specificity of pathogens through field observations and inoculation tests.
5) Obtain export permit and ship inoculum of at least one pathogen to Hawaii Department of Agriculture.

The pathology student working on this project will first travel to Vicosa, Brazil to receive training in methods to isolate and propagate *Coccodiella* and *Ditylenchus* in the laboratory of Dr. Robert Barreto. Upon returning to Costa Rica she will establish field studies at sites already known from previous surveys. In particular, sites near Turrialba (approximately 3 hours from the Univ. Costa Rica) have been found to have the greatest abundance of pathogens, including *Coccodiella* and *Ditylenchus*.

Field collected pathogens will be returned to San Jose for propagation in the university greenhouse. A mist chamber is available for maintaining high humidity. Potted miconia plants of Costa Rican and Hawaiian origin are being grown at the university and will be available for infection. Greenhouse conditions will be monitored daily and status of infections and plant health recorded at least weekly. Appropriate uninfested control plants will be maintained and monitored similarly. Our pathogens of interest are obligate on host plants, so we expect most work will be done outside the laboratory. However, a plant pathology laboratory in the Univ. Costa Rica School of Agronomy has been contacted and is willing to provide assistance should specialized facilities be needed.

To evaluate pathogen host range, at field sites near pathogen-infected *Miconia calvescens*, other melastomes, including *Miconia* species, will be monitored for symptoms. Host specificity also will be tested by inoculating selected plant species at field sites and monitoring for

The project will be supervised by Tracy Johnson (research entomologist, USDA Forest Service, Volcano), in collaboration with Robert Barreto, plant pathologist at Universidade Federal de Vicosa, Brazil. Dr. Johnson has overseen foreign and Hawaii-based development and evaluation of insects for biocontrol of miconia and other forest weeds over the past five years, and has 20 years of research experience in insect-plant interactions. Dr. Barreto has led research in exploration and evaluation of miconia pathogens for Hawaii for over 10 years. Supervision will be maintained by frequent emails and phone calls as well as travel between Costa Rica and Brazil (1 trip by the student, and a reciprocal trip by Barreto). Johnson will travel to Costa Rica in May 2006 to evaluate progress directly.

Paul Hanson, professor of entomology at Universidad de Costa Rica, and Edgar Rojas, miconia project manager at UCR, supervise a team of students and technicians conducting insect studies in that country. Insect team members will provide assistance to pathogen studies when needed and make available a 4wd vehicle dedicated to miconia fieldwork. A post-doctoral entomologist hired with previously awarded HISC funds will also be available to provide oversight and guidance for this project.

Eloise Killgore, plant pathologist at Hawaii Department of Agriculture, will receive pathogens shipped under permit from Costa Rica, and will further evaluate pathogens in quarantine in Honolulu.

Budget

	HISC funds requested	Forest Service match	German (DAAD) match	USGS match
Stipend for German student, travel to Costa Rica			5,000	
Stipend for student assistant at Univ. Costa Rica	1,500			
Project oversight, technical assistance, data analysis		5,000		3,000
Supplies (petris, slides, pots, soil)	100			
Transportation (4wd vehicle fuel and maintenance)	3,000			
International travel (1 trip, 2 weeks Costa Rica – Brazil by student)	2,000			
PCSU direct charge (5%)	367			
Total direct costs	6,967			
Indirect costs (FUNDEVI – Univ. Costa Rica 10%)	733			
Total	$7,700	$5,000	$5,000	$3,000

Literature Cited

Barreto, R.W., C.D.S. Seixas, E. Killgore and D.E. Gardner. 2005. Mycobiota of *Miconia calvescens* and related species from the neotropics, with particular reference to potential biocontrol agents. Pacific Cooperative Studies Unit, University of Hawai'i at Manoa. 24 pp. (Technical Report 132).

Huffaker, C.B., and P.S. Messenger. 1976. Theory and practice of biological control. Academic Press, New York.

Killgore, E.M. 2002. Biological control potential of *Miconia calvescens* using three fungal pathogens. Pp. 45-52 in: Proceedings of a workshop on biological control of invasive

infection at the specific points of inoculation. Nearby asymptomatic *Miconia calvescens* plants will be treated also to check viability of inoculum.

University of Costa Rica scientists will apply for permits to export pathogen inoculum to Hawaii. (USDA APHIS import permit will be maintained by Hawaii Department of Agriculture). Shipping methods will be tested in mock-up before actual shipments. We will utilize the carrier DHL, which has successfully delivered insect agents from Costa Rica for quarantine studies in Hawaii. At the conclusion of this project at least one of the pathogens of interest will be shipped to Hawaii for quarantine studies at the plant pathogen containment facility of the Hawaii Department of Agriculture. The choice of pathogen will depend on outcome of our propagation efforts, other observations, and priorities identified by HDOA scientist Eloise Killgore.

Schedule of activities

Month	Activity
Mar 2006	Student training at pathology lab in Brazil (project start)
Mar-May	Collect pathogens from field sites in Costa Rica
Apr-Aug	Propagate and evaluate pathogens on greenhouse plants at Univ. Costa Rica
May-Jul	Test specificity by inoculating selected melastomes in field
Jun-Jul	Obtain export permit, test shipping methods
Aug	Ship pathogen to Hawaii Dept. Agric.
Aug	Write-up (project completion)

Deliverables

Although we cannot predict with certainty that pathotypes suitable for infecting Hawaiian miconia will be found in Costa Rica, the similarity of biotypes of *Miconia calvescens* from Costa Rica and Hawaii suggests that chances are greatly improved in comparison with pathotypes from Brazil. This project will at least rigorously test the suitability of Costa Rican pathotypes of *Coccodiella* and *Ditylenchus* for biocontrol of miconia. There is a reasonably high likelihood that this project will deliver a new miconia pathogen with great potential to Hawaii Dept of Agriculture.

The written report produced at the end of this project will detail results of field and greenhouse tests evaluating specificity and impacts of these pathogens. In addition, results will be written for publication in an international journal such as Biological Control.

Personnel and Partners

Work will be conducted by a German exchange student, Anna Dietrich, based at the Universidad de Costa Rica. Ms. Dietrich will be working on the project as part of her studies at University of Hanover. She has professional interests in plant pathology and previous experience studying miconia pathogens as a volunteer at the Univ. of Costa Rica. Her internship in Costa Rica will be partially funded through the German Academic Exchange Service (DAAD). Dietrich will be assisted in the field and lab by a student assistant from Univ. of Costa Rica.

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Medeiros, A.C., L.L. Loope, P. Conant, and S. McElvaney. 1997. Status, Ecology, and Management of the Invasive Plant, Miconia calvescens DC (Melastomataceae) in the Hawaiian Islands. Records of the Hawaii Biological Survey for 1996. Bishop Museum Occasional Papers 48: 23-36.

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Picanço, M.C, R.W. Barreto, E.G. Fidelis, A.A. Semeão, J.F. Rosado, S.C. Moreno, E.C. de Barros, G.A. Silva and T. Johnson. 2005. Biological control of *Miconia calvescens* by phytophagous arthropods. Technical Report 134, Pacific Cooperative Studies Unit, University of Hawaii at Manoa.

Seixas, C.D.S., R.W. Barreto, J.L. Bezerra and J. David. 2005. Mycoparasites of *Coccodiella miconiae* (Ascomycota: Phyllachoraceae), a potential biocontrol agent for *Miconia calvescens* (Melastomataceae). Pacific Cooperative Studies Unit, University of Hawai'i at Manoa. 20 pp. (Technical Report 133).

Smith, C.W. 2002. Forest pest biological control in Hawaii. Pp. 91-102 in: Proceedings of a workshop on biological control of invasive plants in native Hawaiian ecosystems, C.W. Smith, J. Denslow, S. Hight (eds.).. Pacific Cooperative Studies Unit, University of Hawai'i at Manoa. 122 pp. (Technical Report 129).

Presented to the Hawaii Invasive Species Council:

Magnitude and Duration of West Nile Viremia in Budgerigars (Parakeets)

Principal Investigator:

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Total Budget:

$6,864

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November 29, 2005

Magnitude and Duration of West Nile Viremia in Budgerigars (Parakeets)

1. Problem Statement:

Since adoption of the West Nile (WN) virus related entry requirements for Hawaii, there has been concern that budgerigars may present greater risk than previously thought for WN virus introduction and that entry requirements should be amended to require a seven-day pre-arrival isolation period for these birds.

During 2003, the Hawaii Department of Agriculture established entry requirements for birds to reduce the likelihood of West Nile virus introduction by the movement of pet and commercial birds into Hawaii. The primary risk reducing measures include: (1) Each shipment must have a valid import permit; (2) Poultry and birds for entry must be quarantined in isolation from mosquitoes in a mosquito-free and mosquito-proof enclosure under the supervision of an accredited veterinarian for a minimum period of seven days; and (3) Birds or poultry must enter Hawaii within thirty-six hours of completion of the seven days isolation.

Based on scientific information available when these entry requirements were adopted (Komar et al, Emerging Infectious Diseases 2003; 3:311-22), chickens, pheasants, quail, chuckers, common pigeons (*Columba livia*), and budgerigars (*Melopsittacus undulates*) that are older than four weeks were excluded from the seven days pre-arrival isolation requirement. Although budgerigars were assigned a reservoir competence index of 0 (incompetent as WN virus reservoirs), only 3 birds were included for WN virus challenge studies.

In the proposed study, 30 budgerigars would be challenged with WN virus-infected mosquitoes and the levels of virus in the blood of the infected birds would be determined daily for 7 days. Finding virus in the blood at levels greater than 10^5 plaque forming units per milliliter suggests budgerigars may be a greater threat for transmitting WN virus to mosquitoes than previously believed. The results of the study will be used to determine if entry requirements for budgerigars should be revised to include a seven-day pre-arrival isolation period.

A similar study was performed on pigeons with 2004 HISC funds by Dr. Richard Bowen, Colorado State University. A copy of the completed challenge study results was previously forwarded to HISC.

2. Methodology or Approach:

The objective is to obtain additional data on the variability in duration and magnitude of viremia in budgerigars (*Melopsittacus undulates*) infected with West Nile virus (WNV) by clinical trial.

A total of 30 seronegative parakeets that appear healthy will be purchased from a pet distributor for use in the study. The birds will be housed 4 per cage within the Animal Disease Laboratory, Colorado State University, under BL3 conditions for at least 5 days prior to challenge with WN virus.

The birds will be challenged with WN virus through the bites of infected *Culex* mosquitoes. Mosquitoes will be hatched and inoculated intrathoracically with 500 to 1000 plaque-forming units (pfu) of the NY99-4132 isolate of WN virus. Birds will be challenged by allowing a group of 4 to 6 mosquitoes to feed for 5 to 10 minutes on the skin over the breast muscle. It is expected that 2 to 4 mosquitoes will feed on each bird. The number of engorged mosquitoes will be recorded for each bird, and two will be frozen and later titrated for WN virus content.

Following challenge, blood will be collected from each bird once daily for 7 days. These samples will be frozen (-80C), and when all samples have been collected, they will be assayed for virus by plaque assay on Vero cells. Briefly, 0.1 ml volumes of serial ten-fold dilutions (10^0 to 10^{-5}) will be inoculated into wells of cells grown in six-well plates. After incubation for 45 minutes, the wells will be overlaid with 2 ml of first overlay medium containing 0.5% agarose. Two days after the primary overlay, a second overlay containing neutral red will be added to each well, and plaques counted on the following three days to allow calculation of virus titers.

Birds will be maintained until 14 days post-challenge, then bled to obtain serum and euthanized. Those sera will be stored in the event that determining antibody response is deemed useful.

Viremia titers will be used to generate confidence intervals for peak viremia and duration of viremia.

3. Schedule of Activities:

Start date of the project is dependent on the availability of staff and the status of other research projects. The project will take about 30 days to complete once parakeets are obtained. The study report will be available with 30 days of project completion.

4. Description of Deliverable Products:

A project report including raw data on peak viremia titers and duration of viremia will be provided. A similar report was provided for the HISC 2004 pigeon study performed by Bowen.

5. Personnel and Partners:

The principal investigator will be Richard Bowen, Professor of Biomedical Sciences, Colorado State University. Bowen has extensive experience in clinical trials using WN virus in a variety of animals, including birds. A list of peer-reviewed publications related to WN virus is provided in the CV (attached).

6. Budget:

Costs to be met by HISC for the proposed WN virus challenge study:

Personnel	
Research Associate, 2 weeks	$1,723
Research Assistant, 40 hours	$480
Fringe benefits	$345
Total personnel	*$2,548*
Bird procurement (30 @ $20 each)	$600
Per diem (20 days at $28 per day)	$560
Serologic screening (40 birds x $3 each)	$120
Virus titrations (30 birds x 7 days x $7 per sample)	$1,470
Mosquito preparation charges	$150
Total direct costs	$5,448
Indirect costs (26%)	*$1,416*
Total budget	$6,864

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Richard Arnold Bowen	Professor of Biomedical Sciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Colorado State University	DVM	1972	Veterinary Medicine
Colorado State University	MS	1976	Reproduction
Colorado State University	PhD	1983	Virology

Institution	Dates	Specialties	Title
Colorado State University	1980 - 1983	Reproduction	Research Associate
Colorado State University	1983 - 1986	Reproduction	Assistant Professor
Colorado State University	1986 – 2002	Reproduction/Virology	Associate Professor
Colorado State University	2002 – date	Infectious Diseases	Professor

A. Selected peer-reviewed publications (in chronological order).

1. Davis BS, Chang GJ, Cropp B, Roehrig JT, Martin DA, Mitchell CJ, Bowen R, Bunning ML: A West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in the enzyme-linked immumosorbant assay. J Virol 75:4040-4047, 2001.
2. Bunning, M.L., R.A. Bowen, C.B. Cropp, K.G. Sullivan, B.S. Davis, N. Komar, M. Godsey, D. Baker, D.L. Hettler, D. A. Holmes, G.J. Biggerstaff and C.J. Mitchell. Experimental infection of horses with West Nile virus. Emerg Infect Dis 2002; 8:380-386.
3. Komar N, Lanciotti R, Bowen R, Langevin S, Bunning M. Detection of West Nile virus in oral and cloacal swabs collected from bird carcasses. Emerg Infect Disease 2002; 8:741-742.
4. Komar, N., Langevin, S., Hinten, S., Nemeth, N., Edwards, E., Hettler, D., Davis B., Bowen, R., Bunning, M. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Disease 2003; 9:311-322.
5. Blitvich BJ, Marlenee NL, Hall RA, Calisher CH, Bowen RA, Roehrig JT, Komar N, Langevin SA, Beaty BJ. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. J Clin Microbiol 41:1041-1047, 2003.
6. Blitvich BJ, Bowen RA, Marlenee NL, Hall RA, Bunning ML, Beaty BJ. Epitope-Blocking Enzyme-linked immunosorbent assays for detection of West Nile virus antibodies in domestic mammals. J Clin Microbiol 2003; 41: 2676-2679.
7. Austgen LE, Bowen RA, Bunning ML, Davis BS, Michell CJ, Chang GJ. Experimental infection of cats and dogs with West Nile virus. Emerg Infect Disease 2004; 10:82-86.
8. Minke JM, Siger L, Karaca K, Austgen L, Gordy P, Bowen R, Renshaw RW, Loosmore S, Audonnet JC, Nordgren B. Recombinant canarypoxvirus vaccine carrying the prM/E genes of West Nile virus protects horses against a West Nile virus-mosquito challenge. Arch Virol 2004; 18:221-230.
9. Siger L, Bowen R, Karaca K, Murray M, Gordy P, Loosmore S, Audonnet J, Nordgren R, Minke JM. Assessment of the efficacy of a single dose of a recombinant vaccine against West Nile Virus in response to natural challenge with West Nile virus-infected mosquitoes in horses. Am J Vet Res 2004; 65:1459-1462.
10. Klenk K, Snow J, Morgan K, Bowen R, Stephens M, Foster F, Gordy P, Beckett S, Komar N, Gubler D, Bunning M. Juvenile American alligators (*Alligator mississippiensis*) as amplifiers of West Nile virus. Emerg Infect Dis 2004; 10:2150-2155.
11. Brault AC, Langevin SA, Bowen RA, Panella NA, Biggerstaff BJ, Miller BR, Komar N. West Nile Viral Strains Exhibit Differential Virulence for American Crows (*Corvus brachyrhynchos*). Emerg Infect Dis 2004; 10:2161-2168.
12. Langevin SA, Brault AC, Panella NA, Bowen RA, Komar N. West Nile virus strains vary in virulence for House Sparrows (*Passer domesticus*) Am J Trop Med Hyg 2005; 72:99-102.
13. Karaca K, Bowen R, Austgen LE, Teehee M, Siger L, Grosenbaugh D, Loosemore L, Audonnet J-C, Nordgren R, Minke J. Recombinant canarypox-vectored West Nile virus (WNV) vaccine protects dogs and cats against a mosquito WNV challenge. Vaccine 2005; 23:3808-3813.
14. Teehee ML, Bunning ML, Stevens S, Bowen RA. Experimental infection of pigs with West Nile virus. Arch Virol 2005; 150:1249-1256.
15. Davis A, Bunning M, Gordy P, Panella N, Blitvich B, Bowen R. Experimental and natural infection of North American bats with West Nile virus. Am J Trop Med Hyg 2005; 73:467-469.

Modification of a multi-pest exclusion fence to be effective against all feral mammals in Hawai'i.

Amount Requested: $11,060

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Problem Statement

Recovery of rare species and restoration of habitat in Hawai'i is largely dependent on sustained reduction of the impacts of detrimental alien species. The complete and long-term removal of non-native mammals from natural areas is highly desirable for conservation of sensitive plants, invertebrates, and vertebrates, but technical barriers to achieving and sustaining such pest-free status have been considered insurmountable except for offshore islands. Large herbivores can currently be fenced out, but because rodents, mongoose and cats cannot, control of these key pests is local and temporary, if attempted at all.

In New Zealand (Aotearoa), by contrast, the development and adoption of pest-proof fencing has spurred the establishment of recovery areas that do not need sustained trapping or poisoning programs once free of mammalian pests (Clapperton & Day 2001). Even species highly vulnerable to predators (e.g. kiwi, tuatara, weta) are surviving and reproducing within these enclosures. Transfer of this technology to Hawai'i, which shares many pests and conservation issues with New Zealand, has been slowed by concerns of cost and of efficacy in the unique Hawaiian environment.

Fence designs developed by the Xcluder™ Pest Proof Fencing Company of New Zealand have been previously shown (Day & MacGibbon 2002) to exclude all rodents and other mammalian pests under New Zealand conditions. Preliminary tests have shown these fences to be effective in Hawai'i with a caveat; they were not mouse-proof. Unless a mouse-proof fence is developed for Hawai'i, removal of larger rodents within enclosures could simply release competitive pressure on mice and not eliminate the effects of rodent predation.

Preliminary Study Results

In 2002, two test fences were erected at The Nature Conservancy's Kona Hema Preserve in South Kona, using funds from the Packard Foundation. Under a contract with the Fish and Wildlife Service, Xcluder built these fences, using their proprietary technology, in the form of octagonal enclosures 8 m in diameter and 2 m high. The basic fence design is marketed as the "Xcluder Kiwi" (Figure 1) but for this purpose is turned "inside out." Animals released into the enclosures attempted to escape, simulating attempted penetration of an exclosure boundary fence.

The test fences were designed to answer three questions:

1. Could the basic fence design be built on lava substrates?
2. What modifications are needed to optimize the design for Hawaiian conditions?
3. Would the fence prevent passage of all pest mammals found in Hawai'i?

The test site, a bulldozed area of 'a'ā lava (Figure 2) was chosen to present a serious challenge to the fence design, being barren of soil and composed of small loose cobbles over solid rock. This type of substrate could be encountered on potential fence alignments, especially on Hawai'i island. During construction it became apparent that the standard fence design, which uses wooden posts rammed into the ground, could not be easily modified for lava. The design was modified in the second pen by the use of

steel components and drilled holes typical of stock fence construction in Hawai'i. The company designated this design the "Xcluder Kona."

A key feature of the Xcluder fence designs is a skirt of fencing mesh that projects outward at or just below ground level, to defeat animals trying to burrow under the fence. Normally grass or other vegetation grows through this mesh and binds it to the soil, but at the Kona test site the skirt design had to be modified. In this modification, most loose cobbles were removed from the mesh zone, the mesh was contoured over the underlying solid rock, and cobbles replaced over the mesh.

Feral cats (*Felis catus*), mongoose (*Herpestes javanicus*), black rats (*Rattus rattus*), mice (*Mus musculus*) and one feral pig (*Sus scrofa*) were released and their behavior observed within the two enclosures. In addition, mouflon sheep (*Ovis musimon*) at a different site were challenged with a section of fence to determine if they could jump over it.

Trial results were as follows:

Species	No. Tested	Escapes
Feral cat	10	0
Mongoose	13	0
Black rat	13	0
Mouse	13	7
Feral Pig	1	0
Mouflon	11	0

All of these species, with the exception of mongoose and mouflon, had been tested repeatedly against the basic fence design in New Zealand and had proven unable to defeat it. Polynesian rats (*Rattus exulans*) and Norway rats (*Rattus norvegicus*) were not available for testing, but Norway rats are less adept at climbing that black rats, and have been previously shown to be defeated by this fence design. Although numbers of individuals tested in Kona were low, observations of behavior suggest that the design parameters of the fence also exceeded the physical abilities of the previously untested species. Mongoose could easily climb the fence but were defeated by the overhanging hood. Mouflon and mouflon-feral sheep hybrids would not attempt to jump the 2 m high fence, possibly because of the visual barrier the hood presented at the top of the fence (D. Goltz, USGS, pers. comm.).

Mice were able to penetrate both fence designs used in Kona, while they had never done so during trials in New Zealand. Close observation determined that mice encountering the fence would burrow down between the cobbles, continue to descend along the mesh skirt below the surface, and slip underneath the skirt where it was poorly sealed against the underlying rock or cobble. The contract term expired before the contractors could test methods to defeat mice in this environment, although a potential modification was devised that could be incorporated into the construction of new fences.

Installation of an Xcluder fence on a rocky region in New Zealand has enabled the company to adapt their design for hard substrates in the time since the Kona trials. Xcluder has offered to send Dr. Tim Day to Hawai'i at company expense to assist in this redesign and trial, as he is their research and development expert.

Methodology

This project will test one, and potentially two mouse-proofing modifications. First, the modification proposed by Xcluder (Figure 3) will be implemented and tested. This will involve re-bedding the mesh skirt of one fence onto size-sorted and leveled rubble and sealing the edge of the mesh to the underlying rock with premixed cement mortar, applied as a powder. Where bedrock is encountered, Ramset® nails will be used to pin the mesh firmly to the rock. This modification will be applied to the steel-framed Xcluder Kona enclosure, and tested for efficacy at blocking mice. In the event that this modification is unsuccessful, or a more efficient technique is devised, the second enclosure will be modified and subsequently tested with mice. Because Polynesian rats are larger than mice, any mouse-proof design should also be proof against this species.

Feral house mice will be collected from the wild and maintained under approved humane conditions. Test mice will be introduced into the enclosure in groups of ten in the afternoon and observed until nightfall. An insulated box will be provided to allow the mice to find shelter and survive potential hypothermia overnight. Mice not present in the morning will be searched for within the enclosure, and if not found they will be scored as escapes. This trial will be repeated at least three times (assuming no escapes) to maximize the chances of mice finding and exploiting gaps or weaknesses in the fence. If escapes occur, we will attempt to observe the route and behavior of escape, and then modify the design accordingly. New modifications will be retested until at least three consecutive groups of mice are completely unsuccessful. Mice will not be reused, in order to maximize the diversity of escape behavior and avoid learning.

Because the high cost of pest-proof fencing may be a significant obstacle to use of the technology in Hawai'i, the time and materials cost of these modifications will be closely measured and documented in the report.

Schedule of activities

- Week of February 20, 2006.
  - Purchase and transport materials to site.
  - Assess pen integrity, repair if needed, construct rock sorter.
  - Expose skirt, sort gravel.
  - Bed and attach skirt.
- Week of February 27.
  - Backfill skirt.
  - Release and monitor mice.
  - Clean site, remove materials and tools.
- Week of March 6.
  - Modify second fence and repeat tests if necessary
- June-July 2006. Complete report, present at HCC meeting.

Deliverable products

1. Written report to cooperating agencies and Xcluder.
2. Oral or poster presentation at Hawai'i Conservation Conference, July 2006

Personnel

PI: Jeff Burgett, U.S. Fish and Wildlife Service, Honolulu HI
Technical Manager: Will Pitt, USDA APHIS, Wildlife Services, Hilo HI
Technical Advisor: Tim Day, Xcluder Pest Proof Fencing Company, Cambridge, New Zealand

Partners

The Nature Conservancy of Hawai'i

Budget

Materials:

• Ramset gun	$200
• Ramset expendables (500 ct)	$200
• mortar mix (15 bags)	$105
• lumber for rock sorter	$40
• wire mesh for rock sorter	$20
• wheelbarrow	$80
• hand tools	$60
• miscellaneous	$200
• gloves, PPE	$100
Subtotal	$1005

Personnel:

PI Salary (16 days @ $236/day)	$3776
PI Travel (2 interisland trips @ $460; incl. airfare, 4WD rental, per diem)	$920
Assistant Salary (23 days @ $233/day)	$5359
Subtotal	$10,055

HISC Project total: $11,060

In-kind Contributions:

Technical Advisor (16 days @ $204/day, travel/per diem $8243)	$11507
Wildlife Services: mousetraps, bait, holding facilities, vehicle, mileage.	$1400
The Nature Conservancy: Field camp facilities (17 days@ $100)	$1700
	$14,607

References:

Day, T.D. and MacGibbon, R.J. 2002. Escape behaviour and physical abilities of vertebrate pests towards electrified and non-electrified fences. Xcluder™ Pest Proof Fencing Company unpublished internal report. 7 pp.
Online at: http://www.xcluder.co.nz/xcluder%20animal%20behaviour.pdf

Clapperton, K.D, and Day, T.D. 2001. Cost-effectiveness of exclusion fencing for stoat and other pest control compared with conventional control. DOC Science Internal Series 14. Department of Conservation, Wellington. 18 pp.

Figure 1. Xcluder Kiwi fence components

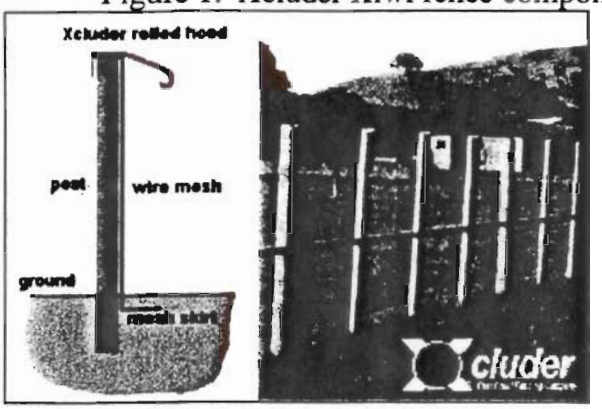

Figure 2. Xcluder Kiwi enclosure at Kona Hema. George Calvert releasing mongoose.

Figure 3. Proposed mouseproofing technique